```
RESULT 19
ADE71415
ID   ADE71415 standard; protein; 810 AA.
XX
AC   ADE71415;
XX
DT   29-JAN-2004  (first entry)
XX
DE   Bacillus sp. KSM-N131 alkaline cellulase Egl-N131b.
XX
KW   Alkaline cellulase; Egl-N131b; detergent; laundry; enzyme.
XX
OS   Bacillus sp. KSM-N131.
XX
PN   WO2003091422-A1.
XX
PD   06-NOV-2003.
XX
PF   25-APR-2003; 2003WO-JP005371.
XX
PR   25-APR-2002; 2002JP-00124474.
XX
PA   (KAOS ) KAO CORP.
XX
PI   Hakamada Y, Ozawa T, Kobayashi T;
XX
DR   WPI; 2003-854397/79.
XX
PT   Mutated alkaline cellulase for use as an enzyme for detergents is
PT   produced by deleting one or more amino acid residue groups from the 343-
PT   to 373-positions in SEQ ID No:1 and then inserting a peptide into the
PT   deletion site.
XX
PS   Disclosure; Fig 1; 31pp; Japanese.
XX
CC   The invention relates to a mutant alkaline cellulase derived from the
CC   Bacillus sp. KSM-S237 alkaline cellulase Egl-237 (ADE71407). The mutant
CC   enzyme is created by deleting one or more amino acid residues between
CC   residues 343-373 of the wild-type enzyme, and then inserting a 2-15
CC   residue peptide into the deletion site. The invention also encompasses a
CC   gene encoding a mutant alkaline cellulase of the invention, and vectors
CC   and host cells comprising a mutant alkaline cellulase-encoding gene. The
CC   mutant alkailine cellulases of the invention have an optimum pH which is
CC   very close to the pH of laundry water (around pH 10.5) and are therefore
CC   useful as enzymes for detergents. Sequences ADE71413-ADE71415 represent
CC   alkaline cellulases from other Bacillus species.
XX
SQ   Sequence 810 AA;

  Query Match             95.0%;  Score 4141;  DB 7;  Length 810;
  Best Local Similarity   95.1%;  Pred. No. 3.5e-274;
  Matches  784;  Conservative   10;  Mismatches   16;  Indels   14;  Gaps    2;

Qy          1 MMLRKKTKQLISSILILVLLLSLFPAALAAEGNTREDNFKHLLGNDNVKRPSEAGALQLQ 60
              ||||||||||           ||  |||||||||||||||||||||||||||||||
Db          1 MMLRKKTKQLGR------------PA--QAEGNTREDNFKHLLGNDNVKRPSEAGALQLQ 46

Qy         61 EVDGQMTLVDQHGEKIQLRGMSTHGLQWFPEILNDNAYKALSNDWDSNMIRLAMYVGENG 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         47 EVDGQMTLVDQHGEKIQLRGMSTHGLQWFPEILNDNAYKALSNDWDSNMIRLAMYVGENG 106

Qy        121 YATNPELIKQRVIDGIELAIENDMYVIVDWHVHAPGDPRDPVYAGAKDFFREIAALYPNN 180
              :|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        107 HATNPELIKQRVIDGIELAIENDMYVIVDWHVHAPGDPRDPVYAGAKDFFREIAALYPNN 166

Qy        181 PHIIYELANEPSSNNNGGAGIPNNEEGWKAVKEYADPIVEMLRKSGNADDNIIIVGSPNW 240
              ||||||||||||||||||||||||||||||||||||:||||||||||||||||||||||
Db        167 PHIIYELANEPSSNNNGGAGIPNNEEGWKAVKEYADPIVQMLRKSGNADDNIIIVGSPNW 226

Qy        241 SQRPDLAADNPIDDHHTMYTVHFYTGSHAASTESYPSETPNSERGNVMSNTRYALENGVA 300
              |||||||||||||||||||||||||||||||||| ||||||||||||||||||||||||
Db        227 SQRPDLAADNPIDDHHTMYTVHFYTGSHAASTESYPPETPNSERGNVMSNTRYALENGVA 286

Qy        301 VFATEWGTSQASGDGGPYFDEADVWIEFLNENNISWANWSLTNKNEVSGAFTPFELGKSN 360
              ||||||||||:||||||||||||||||||||||||||||||||||||||||||||||||
Db        287 VFATEWGTSQANGDGGPYFDEADVWIEFLNENNISWANWSLTNKNEVSGAFTPFELGKSN 346

Qy        361 ATNLDPGPDHVWAPEELSLSGEYVRARIKGVNYEPIDRTKYTKVLWDFNDGTKQGFGVNS 420
              ||:|||||| || ||||||||||||||||||||||||||||||||||||||||||||||||
Db        347 ATSLDPGPDQVWVPEELSLSGEYVRARIKGVNYEPIDRTKYTKVLWDFNDGTKQGFGVNS 406

Qy        421 ESPNKELIAVDNENNTLKVSGLDVSNDVSDGNFWANARLSANGWGKSVDILGAEKLTMDV 480
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        407 DSPNKELIAVDNENNTLKVSGLDVSNDVSDGNFWANARLSANGWGKSVDILGAEKLTMDV 466

Qy        481 IVDEPTTVAIAAIPQSSKSGWANPERAVRVNAEDFVQQTDGKYKAGLTITGEDAPNLKNI 540
              ||||||||||||||||||||||||||||||||||||||||||||||||||:|: |
Db        467 IVDEPTTVAIAAIPQSSKSGWANPERAVRVNAEDFVQQTDGKYKAGLTITGEDAPSLEAI 526

Qy        541 AFHEEDNNMNNIILFVGTDAADVIYLDNIKVIGTEVEIPVVHDPKGEAVLPSVFEDGTRQ 600
              | | |:  :||||||||: ||||||| |||| ||||||||||||||||||||||||||
Db        527 AMHAENYTINNIILEVGTEGADVIYLDTIKVIGPEVEIPVVHDPKGEAVLPSVFEDGTRQ 586

Qy        601 GWDWAGESGVKTALTIEEANGSNALSWEFGYPEVKPSDNWATAPRLDFWKSDLVRGENDY 660
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        587 GWDWAGESGVKTALTIEEANGSNALSWEFGYPEVKPSDNWATAPRLDFWKSDLVRGENDY 646

Qy        661 VAPDFYLDPVRATEGAMNINLVFQPPTNGYWVQAPKTYTINFDELEEANQVNGLYHYEVK 720
              | ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        647 VTFDFYLDPVRATEGAMNINLVFQPPTNGYWVQAPKTYTINFDELEEANQVNGLYHYEVK 706

Qy        721 INVRDITNIQDDTLLRNMMIIFADVESDFAGRVFVDNVRFEGAATTEPVEPEPVDPGEET 780
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        707 INVRDITNIQDDTLLRNMMIIFADVESDFAGRVFVDNVRFEGAATTEPVEPEPVDPGEET 766

Qy        781 PPVDEKEAKKEQKEAEKEEKEAVKEEKKEAKEEKKAVKNEAKKK 824
              ||||||||||||||||||||||||||||||||||:|||| |||
Db        767 PPVDEKEAKKEQKEAEKEEKEAVKEEKKEAKEEKKAIKNEATKK 810


RESULT 20
AAG80267
ID   AAG80267 standard; protein; 813 AA.
XX
AC   AAG80267;
XX
DT   08-FEB-2002  (first entry)
XX
DE   Bacillus sp alkaline cellulase N131b.
XX
KW   Alkaline cellulase; N131b; textile; detergent; treating agent.
XX
OS   Bacillus sp.
XX
FH   Key             Location/Qualifiers
FT   Misc-difference 12
FT                   /note= "Encoded by TGA"
XX
PN   JP2001231569-A.
XX
PD   28-AUG-2001.
XX
PF   24-FEB-2000; 2000JP-00047237.
XX
PR   24-FEB-2000; 2000JP-00047237.
XX
PA   (KAOS ) KAO CORP.
XX
DR   WPI; 2002-029359/04.
DR   N-PSDB; AAI69288.
XX
PT   Alkaline cellulase gene useful for the preparation of an alkaline
PT   cellulase useful as a textile detergent and a textile treating agent.
XX
PS   Example 6; Page 9-11; 22pp; Japanese.
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XX
CC   This invention describes a novel alkaline cellulase gene from a Bacillus
CC   sp. The alkaline cellulase gene is used for the preparation of an
CC   alkaline cellulase useful as a textile detergent and a textile treating
CC   agent. This sequence represents the Bacillus sp. alkaline cellulase N131b
CC   described in the method of the invention
XX
SQ   Sequence 813 AA;

  Query Match             94.6%;  Score 4123;  DB 5;  Length 813;
  Best Local Similarity   97.1%;  Pred. No. 6e-273;
  Matches  774;  Conservative   10;  Mismatches   13;  Indels    0;  Gaps    0;

Qy         28 LAAEGNTREDNFKHLLGNDNVKRPSEAGALQLQEVDGQMTLVDQHGEKIQLRGMSTHGLQ 87
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         17 LAAEGNTREDNFKHLLGNDNVKRPSEAGALQLQEVDGQMTLVDQHGEKIQLRGMSTHGLQ 76

Qy         88 WFPEILNDNAYKALSNDWDSNMIRLAMYVGENGYATNPELIKQRVIDGIELAIENDMYVI 147
              ||||||||||||||||||||||||||||||:||||||||||||||||||||||||||||
Db         77 WFPEILNDNAYKALSNDWDSNMIRLAMYVGENGHATNPELIKQRVIDGIELAIENDMYVI 136

Qy        148 VDWHVHAPGDPRDPVYAGAKDFFREIAALYPNNPHIIYELANEPSSNNNGGAGIPNNEEG 207
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        137 VDWHVHAPGDPRDPVYAGAKDFFREIAALYPNNPHIIYELANEPSSNNNGGAGIPNNEEG 196

Qy        208 WKAVKEYADPIVEMLRKSGNADDNIIIVGSPNWSQRPDLAADNPIDDHHTMYTVHFYTGS 267
              ||||||||||:||||||||||||||||||||||||||||||||||||||||||||||||
Db        197 WKAVKEYADPIVQMLRKSGNADDNIIIVGSPNWSQRPDLAADNPIDDHHTMYTVHFYTGS 256

Qy        268 HAASTESYPSETPNSERGNVMSNTRYALENGVAVFATEWGTSQASGDGGPYFDEADVWIE 327
              |||||||||| ||||||||||||||||||||||||||||||:||||||||||||||||||
Db        257 HAASTESYPPETPNSERGNVMSNTRYALENGVAVFATEWGTSQANGDGGPYFDEADVWIE 316

Qy        328 FLNENNISWANWSLTNKNEVSGAFTPFELGKSNATNLDPGPDHVWAPEELSLSGEYVRAR 387
              |||||||||||||||||||||||||||||||||||:|||||||||||||||||||||||||
Db        317 FLNENNISWANWSLTNKNEVSGAFTPFELGKSNATSLDPGPDQVWVPEELSLSGEYVRAR 376

Qy        388 IKGVNYEPIDRTKYTKVLWDFNDGTKQGFGVNSDSPNKELIAVDNENNTLKVSGLDVSND 447
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        377 IKGVNYEPIDRTKYTKVLWDFNDGTKQGFGVNSDSPNKELIAVDNENNTLKVSGLDVSND 436

Qy        448 VSDGNFWANARLSANGWGKSVDILGAEKLTMDVIVDEPTTVAIAAIPQSSKSGWANPERA 507
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        437 VSDGNFWANARLSANGWGKSVDILGAEKLTMDVIVDEPTTVAIAAIPQSSKSGWANPERA 496

Qy        508 VRVNAEDFVQQTDGKYKAGLTITGEDAPNLKNIAFHEEDNNMNNIILFVGTDAADVIYLD 567
              |||||||||||||||||||||||||||:|: || | |:  :|||||||||: |||||||
Db        497 VRVNAEDFVQQTDGKYKAGLTITGEDAPSLEAIAMHAENYTINNIILFVGTEGADVIYLD 556

Qy        568 NIKVIGTEVEIPVVHDPKGEAVLPSVFEDGTRQGWDWAGESGVKTALTIEEANGSNALSW 627
              |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        557 TIKVIGPEVEIPVVHDPKGEAVLPSVFEDGTRQGWDWAGESGVKTALTIEEANGSNALSW 616

Qy        628 EFGYPEVKPSDNWATAPRLDFWKSDLVRGENDYVAFDFYLDPVRATEGAMNINLVFQPPT 687
              |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        617 EFGYPEVKPSDNWATAPRLDFWKSDLVRGENDYVTFDFYLDPVRATEGAMNINLVFQPPT 676

Qy        688 NGYWVQAPKTYTINFDELEEANQVNGLYHYEVKINVRDITNIQDDTLLRNMMIIFADVES 747
              |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        677 NGYWVQAPKTYTINFDELEEANQVNGLYHYEVKINVRDITNIQDDTLLRNMMIIFADVES 736

Qy        748 DFAGRVFVDNVRFEGAATTEPVEPEPVDPGEETPPVDEKEAKKEQKEAEKEEKEAVKEEK 807
              |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        737 DFAGRVFVDNVRFEGAATTEPVEPEPVDPGEETPPVDEKEAKKEQKEAEKEEKEAVKEEK 796

Qy        808 KEAKEEKKAVKNEAKK 824
              |||||||||:|||| ||
Db        797 KEAKEEKKAIKNEATK 813


RESULT 21
ABG76403
ID   ABG76403 standard; protein; 773 AA.
XX
AC   ABG76403;
XX
DT   23-OCT-2003  (revised)
DT   07-MAY-2003  (first entry)
XX
DE   Bacillus sp. endo-beta-1,4-glucanase.
XX
KW   Enzyme; endo-beta-1,4-glucanase; detergent; textile finishing process;
KW   oil industry; biomass degradation; laundry; stone washing; EC 3.2.1.4;
KW   pulp processing; animal feed.
XX
OS   Bacillus sp; AA349 strain DSM 12648.
XX
FH   Key             Location/Qualifiers
FT   Misc-difference 62
FT                   /note= "Encoded by GAR"
FT   Binding-site    340. .540
FT                   /label= Cellulase_binding_site
FT                   /note= "This site is claimed in claim 24"
XX
PN   WO200299091-A2.
XX
PD   12-DEC-2002.
XX
PF   06-JUN-2002; 2002WO-DK000381.
XX
PR   06-JUN-2001; 2001DK-00000879.
XX
PA   (NOVO ) NOVOZYMES AS.
XX
PI   Outtrup H,  Schuelein M,  Eskelund ME,  Gibson K;
XX
DR   WPI; 2003-256232/25.
DR   N-PSDB; ABX11841.
XX
PT   New enzyme exhibiting endo-beta-1,4-glucanase activity, useful in
PT   detergent compositions, oil industry textile finishing processes, biomass
PT   degradation, laundry, and stone washing.
XX
PS   Claim 1; Page 45-48; 51pp; English.
XX
CC   The invention relates to an enzyme exhibiting endo-beta-1,4-glucanase
CC   activity (EC 3.2.1.4), comprising: (a) a polypeptide encoded by the DNA
CC   sequence appearing as ABX11841; (b) a polypeptide produced by culturing a
CC   cell comprising the DNA sequence under conditions where the DNA sequence
CC   is expressed; (c) an endo-beta-1,4-glucanase enzyme having at least 97%
CC   sequence identity to the amino acid sequence appearing as ABG76403; or
CC   (d) a polypeptide having endo-beta-1,4-glucanase activity that is encoded
CC   by a polynucleotide that hybridizes to the DNA under hybridisation
CC   conditions comprising 5X SSC at 45 plusoC and washing conditions
CC   comprising 2X SC at 60 plusoC. Also included are the encoding DNA
CC   sequence, a polynucleotide construct comprising any of the DNA sequence,
CC   an expression vector (comprising the following operably linked elements:
CC   a transcription promoter, a DNA segment encoding the enzyme and a
CC   transcription terminator), a cultured cell comprising the vector and
CC   expressing the enzyme, a method for degradation of cellulose-containing
CC   biomass that is treated with the enzyme or enzyme composition cited above
CC   and a hybrid endo-glucanase (exhibiting endo- beta-1,4-glucanase activity
CC   comprising the cellulase binding domain, CBD, of the enzyme , and a
CC   catalytic domain (CAD) from sources other than Bacillus sp. AA349 strain
CC   DSM12648). The enzymes are useful in detergent composition, textile
CC   finishing processes, oil industry, biomass degradation, laundry and stone
CC   washing. The invention provides enzymes having substantial beta-1,4-
CC   glucanase activity under slightly acid to alkaline conditions and
CC   improved performance in pulp processing, textile treatment, laundry
CC   processes, extraction processes or in animal feed. The present sequence
CC   represents the endo-beta-1,4-glucanase. (Updated on 23-OCT-2003 to
CC   standardise OS field)
XX
SQ   Sequence 773 AA;

  Query Match             93.1%;  Score 4059;  DB 6;  Length 773;
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MACHINE-ASSISTED TRANSLATION (MAT):

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(54)【発明の名称】 アルカリセルラーゼ遺伝子	**(54)[TITLE OF THE INVENTION]** Alkali cellulase gene
(51)【国際特許分類第７版】 C12N 15/09 ZNA 1/00 1/15 1/19 1/21 5/10 9/42	**(51)[IPC 7]** C12N 15/09 ZNA 1/00 1/15 1/19 1/21 5/10 9/42
【ＦＩ】 C12N 1/00 T 1/15 1/19	**[FI]** C12N 1/00 T 1/15 1/19

1/21		1/21	
9/42		9/42	
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【テーマコード（参考）】
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4B050 CC03 DD02 LL04
4B065 AA15X AA15Y AB01 CA57

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4B024 BA11 CA04 CA09 DA07 EA04 GA11 GA19 GA27 HA01 HA19
4B050 CC03 DD02 LL04
4B065 AA15X AA15Y AB01 BA02 BA22 CA31 CA57

BA02 BA22 CA31 CA57

(57)【要約】
（修正有）

(57)[ABSTRACT OF THE DISCLOSURE]
(Amendments Included)

【解決手段】
特定の配列を有する２種類のアミノ酸配列のいずれか、又は該アミノ酸配列の１若しくは数個のアミノ酸が欠失、置換若しくは付加されたアミノ酸配列をコードするアルカリセルラーゼ遺伝子、組換えベクター及び形質転換体。

[PROBLEM TO BE SOLVED]
The alkali cellulase gene, recombinant vector, and transformed body which code the amino acid sequence of which 1 of either of two kinds of amino acid sequences which has a specific sequence, or this amino acid sequence, or some amino acids were deleted, substituted or added.

【効果】
この遺伝子を用いて衣料用洗剤、繊維処理剤等として有用なアルカリセルラーゼを単一且つ大量に生産することが可能である。

[ADVANTAGE]
Alkali cellulase useful as the detergent for garments, a fiber processing agent, etc. can be produced individually and in large quantities using this gene.

【特許請求の範囲】

[CLAIMS]

【請求項１】
配列番号１若しくは２に示すアミノ酸配列、又は該アミノ酸配列の１若しくは数個のアミノ酸が欠失、置換若しくは付加されたアミノ酸配列をコードするアルカリセルラーゼ遺伝子。

[CLAIM 1]
The alkali cellulase gene which codes the amino acid sequence of which the amino acid sequence shown in sequence number 1 or 2, 1 of this amino acid sequence, or some amino acids were deleted, substituted or added.

【請求項２】
配列番号３若しくは４に示す塩基配列、又は該塩基配列の１若しくは数個の塩基が欠失、置

[CLAIM 2]
The alkali cellulase gene which has the base acid sequence of which the base sequence shown in sequence number 3 or 4, 1 of this

換若しくは付加された塩基酸配列を有するアルカリセルラーゼ遺伝子。

base sequence, or some bases were deleted, substituted or added.

【請求項３】
請求項１又は２記載の遺伝子を含む組換えベクター。

[CLAIM 3]
The recombinant vector containing the gene of Claim 1 or 2.

【請求項４】
請求項３記載の組換えベクターを含む形質転換体。

[CLAIM 4]
The transformed body containing the recombinant vector of Claim 3.

【請求項５】
宿主が微生物である請求項４記載の形質転換体。

[CLAIM 5]
The transformed body of Claim 4 whose host is microorganisms.

【請求項６】
請求項４又は５に記載の形質転換体を培養することを特徴とするアルカリセルラーゼの製造法。

[CLAIM 6]
A production of the alkali cellulase, which cultivates the transformed body of Claim 4 or 5.

【発明の詳細な説明】

[DETAILED DESCRIPTION OF THE INVENTION]

【０００１】

[0001]

【発明の属する技術分野】
本発明は、洗剤用酵素として有用なアルカリセルラーゼをコードする遺伝子に関する。

[TECHNICAL FIELD OF THE INVENTION]
This invention relates to the gene which codes alkali cellulase useful as an enzyme for detergents.

【０００２】

[0002]

【従来の技術】
セルロースは植物細胞壁の主成

[PRIOR ART]
A cellulose is the principal component of a

分で、衣料、紙、建築材料等に有効利用されるバイオマスの代表的存在である。セルロースはグルコースが直鎖状にβ－１，４結合した巨大分子であるため、分解によって燃料物質やより高付加価値の代謝物質に変換が可能である。そのためセルロースを分解する酵素として、セルラーゼ及びその反応産物の有効利用に関する研究が多岐に行われている。これらの研究対象となるセルラーゼは、一般に、中酸性に最適反応ｐＨを有し、結晶性セルロースを良好に分解できる真菌類や嫌気性細菌由来の酵素が中心となっている。

plant-cell wall, and is a typical presence of the biomass used effectively for garments, paper, a building material, etc.
Since the glucose is the macromolecule which carried out the (beta)-1,4 connection linear, the conversion of a cellulose is possible for the fuel matter or a more nearly high-value-added metabolite with a degradation.
Therefore, as an enzyme which degrades a cellulose, research on an effective usage of cellulase and its reaction production is done variably.
Generally the cellulase used as these candidates for research has the optimal reaction pH into the in acidity, the enzyme derived from fungi or the anaerophyte which can degrade a crystalline cellulose good has taken the lead.

【０００３】

一方、掘越（特公昭５０－２８５１５号公報、Horikoshi & Akiba, Alkalophilic Microorganisms, Springer, Berlin, 1982）によって好アルカリ性バチルス属細菌由来のアルカリセルラーゼが見出されて以来、セルラーゼの衣料用重質洗剤への応用が可能となった。その後、実際に好アルカリ性バチルス属細菌の生産するアルカリセルラーゼ（特公昭６０－２３１５８号公報、特公平６－０３０５７８号公報、米国特許第４９４５０５３号等）が衣料用洗剤へ配合されるに至った。これ

[0003]

On the other hand, since the alkali cellulase derived from alkali-loving Bacillus bacteria was discovered by Horikoshi (Japanese Patent Publication No. 50-28515, Horikoshi & Akiba, Alkalophilic Microorganisms, Springer, Berlin, 1982), it has become applicable to the heavy duty detergent for garments of cellulase.
After that, the alkali cellulase (Japanese Patent Publication No. 60-23158, the Japanese Patent Publication No. 6-030578, US Patent 4945053 grade) which alkali-loving Bacillus bacteria actually produce came to be mixed with the detergent for garments.
The cellulase blending detergent derived from fungi also comes to be marketed after this, it has established the status as an enzyme for

以降、真菌類由来のセルラーゼ配合洗剤も上市されるようになり、プロテアーゼ、リパーゼ、アミラーゼと並ぶ洗剤用酵素としての地位を確立してきた。

detergents on a par with the protease, the lipase, and the amylase.

【０００４】

さらに近年、遺伝子工学の発展に伴い、洗剤用酵素の生産も遺伝子組換えにより大量生産されるようになっている。アルカリセルラーゼについても既に数多くの遺伝子についてクローニング、塩基配列の決定がなされ、実生産に用いられている例もある。

[0004]

Furthermore, production of the enzyme for detergents is also mass-produced more gene recombinant with development of genetic engineering in recent years.
As for alkali cellulase, the decision of a cloning and a base sequence about many genes has already been done, there are examples of actual production.

【０００５】

[0005]

【発明が解決しようとする課題】

本発明の目的は、洗剤用酵素として有用なアルカリセルラーゼをコードする遺伝子及びその遺伝子を用いた大量かつ単一のアルカリセルラーゼを製造する方法を確立することにある。

[PROBLEM TO BE SOLVED BY THE INVENTION]

There is objective of the invention in establishing the gene which codes alkali cellulase useful as an enzyme for detergents, and the method of manufacturing the extensive and single alkali cellulase using the gene.

【０００６】

[0006]

【課題を解決するための手段】

本発明者らは、自然界からアルカリセルラーゼ生産菌のスクリーニングを行ったところ、目的に適う酵素を生産する微生物を見出し、さらに当該微生物から

[MEANS TO SOLVE THE PROBLEM]

When the present inventors performs a screening of an alkali cellulase producing microbe from nature, he discovers the microorganisms which produce the enzyme which suits the objective, furthermore, by

アルカリセルラーゼをコードする遺伝子をクローン化することにより、本発明を完成した。

carrying out the cloning of the gene which codes alkali cellulase from said microorganisms, it perfected this invention.

【０００７】

本発明は、配列番号１若しくは２に示すアミノ酸配列、又は該アミノ酸配列の１若しくは数個のアミノ酸が欠失、置換若しくは付加されたアミノ酸配列をコードするアルカリセルラーゼ遺伝子を提供するものである。また、本発明は、配列番号３若しくは４に示す塩基配列、又は該塩基配列の１若しくは数個の塩基が欠失、置換若しくは付加された塩基配列を有するアルカリセルラーゼ遺伝子を提供するものである。また、本発明は、上記のアルカリセルラーゼ遺伝子を含む組換えベクター、及び該組換えベクターを含む形質転換体を提供するものである。また、本発明は、上記の形質転換体を培養することを特徴とするアルカリセルラーゼの製造法を提供するものである。

[0007]

This invention provides the alkali cellulase gene which codes the amino acid sequence by which the amino acid sequence shown in sequence number 1 or 2, 1 of this amino acid sequence, or some amino acids were delete, substitute or added.

Moreover, this invention provides the alkali cellulase gene which has the base sequence by which the base sequence shown in sequence number 3 or 4, 1 of this base sequence, or some bases were delete, substitute or added.

Moreover, this invention provides the recombinant vector containing the above-mentioned alkali cellulase gene, and the transformed body containing this recombinant vector.

Moreover, this invention cultivates the above-mentioned transformed body.

It provides the production of the alkali cellulase characterized by the above-mentioned.

【０００８】

[0008]

【発明の実施の形態】

本発明の遺伝子は、配列番号１若しくは２に示すアミノ酸配列、又は該アミノ酸配列の１若しくは数個のアミノ酸が欠失、置換若しくは付加されたアミノ

[EMBODIMENT OF THE INVENTION]

The gene of this invention has the sequence which codes the amino acid sequence by which the amino acid sequence shown in sequence number 1 or 2, 1 of this amino acid sequence, or some amino acids were delete, substitute or

酸配列をコードする配列を有する。アルカリセルラーゼ活性を失わない限り、該アミノ酸配列中のアミノ酸の欠失、置換又は付加（以下、変異ということがある）は特に制限されない。また、配列番号１又は２に示した成熟酵素のアミノ酸配列におけるアミノ末端には、１～数個のアミノ酸が付加、欠失、置換していてもよい。

added.
Unless alkali cellulase activity is lost, deletion of the amino acid in this amino acid sequence, substitution, or addition (it may call it variation hereafter) in particular is not limited.
Moreover, as for the amino terminus in the amino acid sequence of the mature enzyme shown in sequence number 1 or 2, one or more amino acids may be added, deleted or replaced.

【０００９】

本発明の配列番号１に示すアルカリセルラーゼ（以下、Ｎ１３１ａセルラーゼと表記する）のアミノ酸配列と従来公知のセルラーゼのアミノ酸配列との相同性を比較すると、Bacillus sp. No.1139 株の生産するセルラーゼ（Fukumoriら、J.Gen. Microbiol., 131, 3339-3345, 1985）との相同性は８１．９％であり、Bacillus sp. KSM-64 株由来のセルラーゼ（Sumitomoら、Biosci. Biotechnol. Biochem., 56, 872-877, 1992）との相同性は８３．６％、Bacillus sp. KSM-S237 株が生産するセルラーゼ（特願平１１－０１３０４９号）との相同性は８６．７％であり、本発明の遺伝子からコードされるＮ１３１ａセルラーゼと最も高い相同性を示したが、完全に一致するものではなかった。このことは、

[0009]

When the homology of the amino acid sequence of alkali cellulase (it shows it as N131a cellulase hereafter) and the amino acid sequence of conventionally well-known cellulase which are shown in sequence number 1 of this invention is compared, the homology with the cellulase (Fukumori et al., J.Gen.Microbiol., 131, 3339-3345, 1985) which a Bacillus sp.No. 1139 strain produces is 81.9%.
The homology with the cellulase (Japanese Patent Application No. 11-013049) in which 237 strain of Bacillus sp.KSM-S produces the homology with the cellulase (Sumitomo et al., Biosci.Biotechnol.Biochem., 56, 872-877, 1992) derived from Bacillus sp.KSM-64 strain 83.6% is 86.7%.
The N131a cellulase coded from the gene of this invention and the highest homology were shown.
However, it was not what is completely in agreement.
This suggests that N131a cellulase is new alkali

Ｎ１３１ａセルラーゼが新規なアルカリセルラーゼであることを示唆するものであり、従って配列番号１に示したアミノ酸配列と最大８７％以上の相同性を有するセルラーゼは本発明に含まれる。

cellulase.
Therefore, the amino acid sequence shown in sequence number 1 and the cellulase which has a maximum of 87 % or more homology are contained in this invention.

【００１０】

次に、本発明の配列番号２に示すアルカリセルラーゼ（以下、Ｎ１３１ｂセルラーゼと表記する）のアミノ酸配列と従来公知のセルラーゼのアミノ酸配列との相同性を比較すると、上記のＮ１３１ａセルラーゼとの相同性は８３．６％、Bacillus sp. No.1139 株の生産するセルラーゼとの相同性は８８．０％、Bacillussp. KSM-64 株由来のセルラーゼとの相同性は９０．９％であった。さらに、Bacillus sp. KSM-S237 株が生産するセルラーゼとの相同性が９４．７％と本発明の遺伝子からコードされるＮ１３１ｂと最も高い相同性を示した。このことは、Ｎ１３１ｂセルラーゼが従来公知のセルラーゼとは完全に一致するものではなく、新規な酵素であることを示唆するものであり、従って配列番号２に示したアミノ酸配列と最大９５％以上の相同性を有するセルラーゼは本発明に含まれる。尚、相同性の検索はＧＥＮＥＮＴＹＸ－Ｃ

[0010]

Next, when the homology of the amino acid sequence of alkali cellulase (it shows it as N131b cellulase hereafter) and the amino acid sequence of conventionally well-known cellulase which are shown in sequence number 2 of this invention was compared, the homology with the cellulase derived from Bacillussp.KSM-64 strain of the homology with the cellulase which, as for the homology with the above-mentioned N131a cellulase, a Bacillus sp.No. 1139 strain produces 83.6% was 90.9% 88.0%.
Furthermore, the homology with the cellulase which 237 strain of Bacillus sp.KSM-S produces showed 94.7%, N131b coded from the gene of this invention, and the highest homology.
N131b cellulase of conventionally well-known cellulase does not correspond completely, and this suggests that it is a new enzyme.
Therefore, the amino acid sequence shown in sequence number 2 and the cellulase which has a maximum of 95 % or more homology are contained in this invention.
In addition, it performed the search of homology with the maximum matching method which used the GENENTYX-CD bio-data software [software-development company make and

Ｄバイオデータソフトウェア［ソフトウェア開発社製、ｖｅｒ．３６］を用いたマキシマムマッチング法にて行った。

ver.36].

【００１１】
本発明のアルカリセルラーゼ遺伝子は、配列番号１若しくは２に示すアミノ酸配列又はその変異体をコードするものであればよいが、配列番号３若しくは４で示される塩基配列、又は該塩基配列の１若しくは数個の塩基が欠失、置換若しくは付加された塩基配列を有するものが好ましい。

[0011]
The alkali cellulase gene of this invention should just code the amino acid sequence shown in sequence number 1 or 2, or its variant. However, what has the base sequence by which the base sequence shown by sequence number 3 or 4, 1 of this base sequence, or some bases were delete, substitute or added is desirable.

【００１２】
本発明のアルカリセルラーゼ遺伝子は、バチルス属に属する微生物、例えば下記の菌学的性質を有するバチルス　エスピー　ＫＳＭ－Ｎ１３１株等からクローン化することができる。
〔バチルス　エスピー　ＫＳＭ－Ｎ１３１株の菌学的性質〕
Ａ．形態学的性質；
（ａ）細胞の形及び大きさ：桿菌（０．６～０．８×２．８～７．２μｍ）

[0012]
The alkali cellulase genes of this invention are the microorganisms belonging to the Bacillus, for example, Bacillus sp which has the following mycological characteristics. It can carry out a cloning from 131 strain of KSM-N etc.
[Mycological characteristics of 131 strain of Bacillus sp KSM-N]
A. Morphological characteristic;
(a) Form and size of cell : Bacillus (0.6-0.8*2.8 to 7.2 micrometer)

（ｂ）多形性：無し
（ｃ）運動性：有り
（ｄ）胞子の形、大きさ、位置、膨潤の有無：楕円形、０．７～１．０×１．０～１．８μｍ、

(b) Polymorphism : nothing
(c) Manoeuverability : be.
(d) Form of spore, size, position, existence of swelling : ellipse form, 0.7-1.0*1.0 to 1.8 micrometer, center semi- end, those with

中央準端、膨潤有り
swelling

（ｅ）グラム染色：陽性
(e) Gram's stain : positive

（ｆ）抗酸性：陰性
(f) Acid-fastness : negativity

【００１３】
[0013]

Ｂ．培養学的性質；
B. Culture study characteristic;

（ａ）一般細菌用液体培地（ｐＨ５．７、培地１）：生育せず
(a) Broth for standard bacteria (pH5.7, medium 1) : don't grow.

（ｂ）一般細菌用液体培地（ｐＨ６．８、培地１）：生育せず
(b) Broth for standard bacteria (pH6.8, medium 1) : don't grow.

（ｃ）一般細菌用寒天培地（ｐＨ６．５、培地２）：生育せず
(c) Agar for standard bacteria (pH6.5, medium 2) : don't grow.

（ｄ）一般細菌用寒天培地（ｐＨ８．５、培地２）：生育する
(d) Agar for standard bacteria (pH8.5, medium 2) : grow.

【００１４】
[0014]

Ｃ．生理学的性質；
C. Physiological characteristic;

（ａ）硝酸塩の還元（培地３）：陽性
(a) Reduction of nitrate (medium 3) : positive

（ｂ）脱窒反応（培地３）：陰性
(b) Denitrification reaction (medium 3) : negativity

（ｃ）ＶＰテスト（培地４）：陰性
(c) VP test (medium 4) : negativity

（ｄ）インドールの生成（培地５）：陰性
(d) Formation of indole (medium 5) : negativity

（ｅ）硫化水素の生成（培地６）：陰性
(e) Formation of hydrogen sulfide (medium 6) : negativity

（ｆ）デンプンの加水分解（培地７）：陽性
(f) Hydrolysis of starch (medium 7) : positive

（ｇ）カゼインの加水分解（培地８）：陰性
(g) Hydrolysis of casein (medium 8) : negativity

（ｈ）ゼラチンの液化（培地９）：陽性
(h) Liquefying of gelatin (medium 9) : positive

- Utilization of 1 citric acid (medium 10) :

（ｉ）クエン酸の利用（培地１０）：陰性
（ｊ）カタラーゼ：陽性
（ｋ）オキシダーゼ（培地１１）：陽性

negativity
(j) Catalase : positive
(k) Oxidase (medium 11) : positive

（ｌ）生育の温度範囲（培地１２）：１３－４２℃、至適範囲：２３－３８℃
（ｍ）生育のｐＨ範囲（培地１３）：ｐＨ７．６－１０．５、至適範囲：ｐＨ９－９．５
（ｎ）生育における酸素の影響（培地１４）：嫌気条件下で微弱だが生育する。
（ｏ）グルコースからのガス産生（培地１５）：陰性

(l) Temperature range of growth (medium 12) : 13 to 42 degree C, optimum range:23-38 degree C
(m) The pH range of growth (medium 13) : pH7.6-10.5, optimum range:pH9-9.5
(n) Influence of the oxygen in growth (medium 14) : on anaerobic conditions, although it is feeble, grow.
(o) Gas production from glucose (medium 15) : negativity

（ｐ）塩化ナトリウム耐性（培地１６）：１０％塩化ナトリウム存在下で生育する。
（ｑ）馬尿酸の加水分解（培地１７）：陰性
（ｒ）４－メチルウンベリフェリル－β－Ｄ－グルクロニド（ＭＵＧ）の加水分解（培地１８）：陰性
（ｓ）糖の利用性（培地１９）：グルコース、アラビノース、キシロース、マンニトール、ガラクトース、シュークロース、マンノース、マルトース、ラクトース、トレハロース、フラクトース、メリビオース、リボース、サリシン、グリセロール、ソルビトール等を炭素源として生育

(p) Sodium chloride resistance (medium 16) : grow in a sodium chloride presence 10%.
(q) Hydrolysis of hippuric acid (medium 17) : negativity
(r) Hydrolysis of 4-methyl umbelliferyl-(beta)-D-glucuronide (MUG) (medium 18) : negativity
(s) Utility of saccharide (medium 19) : it can grow the glucose, the arabinose, the xylose, a mannitol, the galactose, sucrose, the mannose, the maltose, a lactose, a trehalose, a fructose, the melibiose, the ribose, the salicin, a glycerol, sorbitol, etc. as a source of a carbon.
It cannot utilize a rhamnose and an inositol as a source of a carbon.

可能である。ラムノース、イノシトールを炭素源として利用できない。

【００１５】
培地１：ニュートリエントブロス（ディフコ）指示量、希塩酸にてｐＨを調整
培地２：ニュートリエントアガー（ディフコ）指示量、炭酸ナトリウムにてｐＨを調整
培地３：ニュートリエントブロス０．８重量%、硝酸カリウム０．１重量%、炭酸ナトリウム０．１重量%（別滅菌）
培地４：バクトペプトン（ディフコ）０．７重量%、塩化ナトリウム０．５重量%、グルコース０．５重量%（別滅菌）、炭酸ナトリウム０．２重量%（別滅菌）

培地５：ＳＩＭ培地（日水製薬）指示量、炭酸ナトリウム０．１重量%（別滅菌）、インドール産生試験用濾紙（日水製薬）
培地６：ＴＳＩ寒天培地（栄研化学）指示量、炭酸ナトリウム０．１重量%（別滅菌）
培地７：バクトペプトン１．５重量%、酵母エキス０．５重量%、可溶性デンプン２．０重量%、リン酸１水素カリウム０．１重量%、硫酸マグネシウム７水塩０．０２重量%、寒天１．５重量%、炭酸ナトリウム０．

[0015]
Medium 1: The amount of nutrient-broth (Difco) commands and the diluted hydrochloric acid adjust pH.
Medium 2: Adjust pH in the amount of nutrient agger (Difco) commands, and the sodium carbonate.
Medium 3: 0.8 weight% of nutrient broth, 0.1 weight% of potassium nitrate, 0.1 weight% (another sterilization) of sodium carbonate
Medium 4: 0.7 weight% (Difco) of bacto peptone, 0.5 weight% of sodium chloride, 0.5 weight% (another sterilization) of glucose, 0.2 weight% (another sterilization) of sodium carbonate

Medium 5: The amount of SIM medium (Nissui Pharmaceuticals) commands, 0.1 weight% (another sterilization) of sodium carbonate, the filter paper for an indole production test (Nissui Pharmaceuticals)
Medium 6: The amount of TSI agar (Eiken Chemical) commands, 0.1 weight% (another sterilization) of sodium carbonate
Medium 7: 1.5 weight% of bacto peptone, 0.5 weight% of yeast extract, 2.0 weight% of soluble starch, 0.1 weight% of phosphoric-acid 1 hydrogen potassium, 0.02 weight% of magnesium-sulfate heptahydride, 1.5 weight% of agar, 0.2 weight% (another sterilization) of

２重量％（別滅菌）
培地８：酵母エキス０．５重量％、グルコース２．０重量％、カゼイン０．５重量％、リン酸１水素カリウム０．１重量％、硫酸マグネシウム７水塩、０．０２重量％、寒天１．５重量％、炭酸ナトリウム０．１重量％（別滅菌）

sodium carbonate
Medium 8: 0.5 weight% of yeast extract, 2.0 weight% of glucose, 0.5 weight% of casein, 0.1 weight% of phosphoric-acid 1 hydrogen potassium, magnesium-sulfate heptahydride, 0.02 weight%, 1.5 weight% of agar, 0.1 weight% (another sterilization) of sodium carbonate

培地９：ニュートリエントブロス０．８重量％、ゼラチン１．２重量％、酵母エキス０．５重量％、炭酸ナトリウム０．２重量％（別滅菌）
培地１０：リン酸１水素アンモニウム０．１重量％、リン酸２水素カリウム０．１重量％、硫酸マグネシウム７水塩、０．０２重量％、クエン酸ナトリウム０．２重量％、寒天１．５重量％、炭酸ナトリウム０．１重量％（別滅菌）
培地１１：チトクロムオキシダーゼ試験濾紙（日水製薬）
培地１２：トリプティケースソイ　ブロス（ＢＢＬ）指示量、炭酸ナトリウム０．１重量％（別滅菌）

Medium 9: 0.8 weight% of nutrient broth, 1.2 weight% of gelatin, 0.5 weight% of yeast extract, 0.2 weight% (another sterilization) of sodium carbonate
Medium 10: 0.1 weight% of phosphoric-acid 1 hydrogen ammoniums, 0.1 weight% of monobasic potassium phosphate, magnesium-sulfate heptahydride, 0.02 weight%, 0.2 weight% of sodium citrate, 1.5 weight% of agar, 0.1 weight% (another sterilization) of sodium carbonate
Medium 11: Cytochrome oxidase test filter paper (Nissui Pharmaceuticals)
Medium 12: Tryptocase soy　The amount of broth (BBL) commands, 0.1 weight% (another sterilization) of sodium carbonate

培地１３：トリプティケースソイ　ブロスに炭酸ナトリウムあるいは水酸化ナトリウムを別滅菌後に添加し、ｐＨを調整
培地１４：アナエロビックアガー（ディフコ）指示量、炭酸ナ

Medium 13: Tryptocase soy　It adds the sodium carbonate or the sodium hydroxide to a broth after another sterilization, it adjusts pH.
Medium 14: The amount of anaerobic agger (Difco) commands, 0.2 weight% (another sterilization) of sodium carbonate

トリウム０．２重量％（別滅菌）
培地１５：バクトペプトン１．０重量％、塩化ナトリウム０．５重量％、グルコース１．０重量％、フェノールレッド０．００２重量％、水酸化ナトリウムにてｐＨを調整
培地１６:バクトトリプトン(ディフコ）０．５重量％、酵母エキス１．５重量％、リン酸１水素カリウム０．３重量％、寒天２．０重量％、グルコース２．０重量％（別滅菌)、塩化ナトリウム０－１６重量％、炭酸ナトリウム０．５重量％（別滅菌）

Medium 15: Adjust pH in 1.0 weight% of bacto peptone, 0.5 weight% of sodium chloride, 1.0 weight% of glucose, 0.002 weight% of phenol red, and the sodium hydroxide.
Medium 16: 0.5 weight% (Difco) of bactotryptons, 1.5 weight% of yeast extract, 0.3 weight% of phosphoric-acid 1 hydrogen potassium, 2.0 weight% of agar, 2.0 weight% (another sterilization) of glucose, 0 to 16 weight% of sodium chloride, 0.5 weight% (another sterilization) of sodium carbonate

培地１７:バクトトリプトン１．０重量％、肉エキス（ディフコ）０．３重量％、酵母エキス０．１重量％、グルコース０．１重量％、リン酸１水素ナトリウム０．５重量％、馬尿酸１．０重量％、炭酸ナトリウム１．０重量％（別滅菌）
培地１８：バクトトリプトース（ディフコ）２．０重量％、塩化ナトリウム０．５重量％、システイン塩酸塩０．１重量％、寒天１．５重量％、ＭＵＧ１００ｐｐｍ（濾過滅菌)、炭酸ナトリウム０．３重量％（別滅菌）
培地１９：硝酸カリウム０．２重量％、リン酸１水素ナトリウム０．２重量％、塩化ナトリウム０．５重量％、硫酸マグネシウム７水塩０．００５重量％、

Medium 17: 1.0 weight% of bactotryptons, 0.3 weight% (Difco) of meat extracts, 0.1 weight% of yeast extract, 0.1 weight% of glucose, 0.5 weight% of phosphoric-acid 1 hydrogen sodium, 1.0 weight% of hippuric acid, 1.0 weight% (another sterilization) of sodium carbonate
Medium 18: 2.0 weight% (Difco) of bacto tryptose, 0.5 weight% of sodium chloride, 0.1 weight% of cystein hydrochloride, 1.5 weight% of agar, MUG100 ppm (filtration sterilization), 0.3 weight% (another sterilization) of sodium carbonate
Medium 19: 0.2 weight% of potassium nitrate, 0.2 weight% of phosphoric-acid 1 hydrogen sodium, 0.5 weight% of sodium chloride, 0.005 weight% of magnesium-sulfate heptahydride, 0.2 volume % of trace amount metal <mixed-liquid SP> *</SP>, 0.2 volume % of vitamin <mixed-liquid SP> **</SP>, the

微量金属混液*０．２容量%、ビタミン混液**０．２容量%、炭酸緩衝液（ｐＨ１０）０．１Ｍ、寒天０．３重量%（別滅菌）、糖類１．０重量%（濾過滅菌）＊、＊＊；Nielsenら、Microbiology, 141, 1745-1761(1995)に準ずる。

carbonic acid buffer (pH10) 0.1M, 0.3 weight% (another sterilization) of agar, 1.0 weight% (filtration sterilization) of sugars
* **;
It applies to Nielsen et al., Microbiology, 141,1745-1761 (1995).

【００１６】

以上、ＫＳＭ－Ｎ１３１株は中性培地に生育しない好アルカリ性細菌であり、且つグラム陽性、カタラーゼ陽性の有胞子桿菌であることから、好アルカリ性バチルス属細菌であると判断された。そこで本菌株の形態学、生理学的性質について、Nielsenらが新たに分類した好アルカリ性バチルス属細菌の記載（Microbiology、141、1745-1761、1995）に準じ比較検討した結果、本菌株はバチルス　シュウドアルカロフィルスに近縁な菌種であると考えられた。しかし、その性質は既知のバチルス　シュウドアルカロフィルスと完全には一致せず、他のバチルス属菌の諸性質とも一致しないため、新規なバチルス属細菌として本菌株を工業技術院生命工学研究所へ、バチルス　エスピー　ＫＳＭ－Ｎ１３１株（ＦＥＲＭＰ－１７４７５）として寄託した。

[0016]

As mentioned above, 131 strain of KSM-N is alkalophilic bacteriums which it does not grow to a neutral medium.
And since it was a gram-positive and catalase electropositive owner spore Bacillus, it was judged that they were alkali-loving Bacillus bacteria.
Then, Nielsen and others did comparison examination about the morphology of this-microbe strain, and a physiological characteristic according to publication (1745-Microbiology, 141, 1761, 1995) of the newly categorized alkali-loving Bacillus bacteria.
As a result, it was thought that this-microbe strain was a microbial species with close relation to a Bacillus shoed alcalophilus.
However, since it is not in agreement with a known Bacillus shoed alcalophilus and completeness and in agreement with the characteristics of several of another Bacillus genus, the characteristic is Bacillus sp to an institute-of-technology biotechnology research laboratory about this-microbe strain as new Bacillus bacteria.　It deposited as 131 strain (FERMP-17475) of KSM-N.

【００１７】
上記のＫＳＭ－Ｎ１３１株からのアルカリセルラーゼ遺伝子のクローニング方法としては、既知の手段、例えばショットガン法、ＰＣＲ法を用いて行うことができる。

[0017]
As the cloning method of the alkali cellulase gene from 131 strain of above-mentioned KSM-N, they are known means, for example, it can carry out using the shotgun method and PCR method.

【００１８】
また、本発明のアルカリセルラーゼ遺伝子を含む組換えベクターを作製するには、宿主内で複製維持が可能で、該酵素を安定に発現させることができ、該遺伝子を安定に保持できるベクターにアルカリセルラーゼ遺伝子を組込めばよい。かかるベクターとしては大腸菌を宿主とする場合、ｐＵＣ１８、ｐＢＲ３２２、ｐＨＹ３００ＰＬＫ等が挙げられ、枯草菌を宿主にする場合、ｐＵＢ１１０、ｐＨＳＰ６４（Sumitomo ら、Biosci. Biotechnol. Biochem., 59, 2172-2175, 1995）、ｐＨＹ３００ＰＬＫ等が挙げられる。

[0018]
Moreover, what is necessary is for duplication maintenance to be possible within the host, and to be able to let this enzyme express stably and just to integrate an alkali cellulase gene in the vector which can maintain this gene stably, in order to produce the recombinant vector containing the alkali cellulase gene of this invention.
When making an Escherichia coli into the host as this vector, pUC18, pBR322, and pHY300PLK etc. are mentioned, when making the Bacillus subtilis into the host, pUB110, pHSP64 (Sumitomo et al., Biosci.Biotechnol.Biochem., 59, 2172-2175, 1995), and pHY300PLK etc. are mentioned.

【００１９】
かくして得られた組換えベクターを用いて宿主菌を形質転換するには、プロトプラスト法、コンピテントセル法、エレクトロポレーション法等を用いて行うことができる。宿主菌としては特に制限されないが、Bacillus 属（枯草菌）等のグラム陽性菌；

[0019]
In order to transform a host microbe using the recombinant vector obtained by the thing which write, and to do, it can carry out using the protoplast method, the competent cell method, the electroporation method, etc.
It does not limit particularly as a host microbe. However, gram positive bacteria, such as a Bacillus genus (Bacillus subtilis);

Escherichia coli（大腸菌）等のグラム陰性菌；Streptomyces 属（放線菌）、Saccharomyces 属（酵母）、Aspergillus 属（カビ）等の真菌が挙げられる。

Gram negative bacteria, such as Escherichia coli (Escherichia coli);
Fungi, such as a Streptomyces genus (actinomycetes), a Saccharomyces genus (yeast), and an Aspergillus genus (fungi), are mentioned.

【００２０】
得られた形質転換体を培養し、当該培養液からアルカリセルラーゼを採取することにより、アルカリセルラーゼを得ることができる。培養は、宿主菌又は形質転換株が資化しうる炭素源、窒素源、金属塩、ビタミン等を含む培地を用いて適当な条件下で行なえばよい。かくして得られた培養液から、一般的な方法によって酵素の採取、精製を行い、凍結乾燥、噴霧乾燥、結晶化等により、所望の酵素形態とすることができる。

[0020]
It cultivates the obtained transformed body, by collecting alkali cellulase from said culture medium, it can obtain alkali cellulase.
What is sufficient is just to perform a culture on suitable conditions using the medium containing the source of a carbon which a host microbe or the transformant can utilize, the source of nitrogen, a metallic salt, a vitamin, etc.
From the culture medium obtained by the thing which write, and to do, it can perform collection of an enzyme, and purification by the general method, and can consider it as the desired enzyme form according to freeze-dried, spray drying, crystallization, etc.

【００２１】

[0021]

【実施例】
実施例１（アルカリセルラーゼ生産菌のスクリーニング）
日本各地の土壌を滅菌水に懸濁したものを８０℃、３０分間熱処理し、以下の組成を有する寒天平板培地に塗布した［２．０重量%カルボキシメチルセルロース（Ａ１０ＭＣ；日本製紙社製）、１．０重量%肉エキス（オキソイド社製）、１．０重量%バ

[EXAMPLES]
Example 1 (screening of an alkali cellulase producing microbe)
It heat-processes 80 degrees C of things which suspended the soil of every place of Japan in the sterilized water for 30 minutes, [2.0-weight% carboxymethylcellulose applied to the agar planar medium which has the following compositions (A10MC;)
The Nippon Paper Industries make, the 1.0-weight% meat extract (oxo id shrine make),

クトペプトン（ディフコ社製）、１．０重量％塩化ナトリウム、０．１重量％リン酸２水素カリウム、０．５重量％炭酸ナトリウム(別滅菌)、０．００５重量％トリパンブルー（別滅菌)]。３０℃の培養器で３日間静置培養し、生育した菌の周辺にカルボキシメチルセルロースの分解に伴う溶解斑が検出されたものについて選抜し、シングルコロニー化を繰り返した。これらの菌株を、２．０重量％ポリペプトンＳ（日本製薬社製)、１．０重量％魚肉エキス（和光純薬社製)、０．１５重量％リン酸１水素カリウム、０．１重量％酵母エキス（ディフコ社製)、０．０７重量％硫酸マグネシウム７水塩、０．１重量％カルボキシメチルセルロース及び０．５重量％炭酸ナトリウム（別滅菌）から成る液体培地を用い、３０℃、３日間振盪培養した。アルカリセルラーゼを生産している菌株を選択し、とりわけ高アルカリ性域で強力な活性を示したセルラーゼ生産菌としてバチルス　エスピー　ＫＳＭ－Ｎ１３１株を取得した。

the 1.0-weight% bacto peptone (made by a Difco company), 1.0-weight% sodium chloride, 0.1-weight% monobasic potassium phosphate, the 0.5-weight% sodium carbonate (another sterilization), and the 0.005-weight% trypan blue (another sterilization)].
It carries out stationary culture for three days by a 30-degree C incubator, it selects about that from which the melting spots accompanying a degradation of carboxymethylcellulose were detected around the grown microbe, it repeated single colony-ization.
It carried out the shaking culture of the 30 degrees C of these strains for three days using the broth which constitutes of the 2.0-weight% polypeptone S (made by NIHON PHARMACEUTICAL CO., LTD.), the 1.0-weight% fish-meat extract (made by a Wako Purechemical KK), 0.15-weight% phosphoric-acid 1 hydrogen potassium, the 0.1-weight% yeast extract (made by a Difco company), 0.07-weight% magnesium-sulfate heptahydride, 0.1-weight% carboxymethylcellulose, and the 0.5-weight% sodium carbonate (another sterilization).
It chooses the strain which produces alkali cellulase, it is Bacillus sp as a cellulase producing microbe which showed activity especially powerful in a high alkaline region. It acquired 131 strain of KSM-N.

【００２２】
実施例２（バチルス　エスピー　ＫＳＭ－Ｎ１３１株のゲノムＤＮＡの調製）
バチルス　エスピー　ＫＳＭ－

[0022]
Example 2 (manufacture of the genome DNA of 131 strain of Bacillus sp KSM-N)
Bacillus sp Using the medium which constitutes of the 2.0-weight% polypeptone S,

Ｎ１３１株の培養は、２．０重量％ポリペプトンＳ、０．１重量％カルボキシセルロース（Ａ１０ＭＣ）、０．１重量％酵母エキス、１重量％魚肉エキス、０．１５重量％リン酸１水素カリウム、０．０７重量％硫酸マグネシウム７水塩、０．５重量％グルタミン酸ナトリウム(別滅菌)及び０．５重量％炭酸ナトリウム（別滅菌）から成る培地を用い、３０℃、４０時間振盪（１２５ｒｐｍ）して行った。得られた培養液約３００ｍＬから遠心分離（１２０００×ｇ、１５分、5℃）により菌体を回収し、この菌体から斉藤・三浦の方法によりゲノムＤＮＡを調製した。

the 0.1-weight% carboxy cellulose (A10MC), the 0.1-weight% yeast extract, the 1-weight% fish-meat extract, 0.15-weight% phosphoric-acid 1 hydrogen potassium, 0.07-weight% magnesium-sulfate heptahydride, the 0.5-weight% sodium glutamate (another sterilization), and the 0.5-weight% sodium carbonate (another sterilization), 30 degrees C, it shook the culture of 131 strain of KSM-N for 40 hours (125 rpm), and performed it.
Centrifugation (12000*g, 15 minutes, 5 degrees C) recovers a microbial cell from about 300 mL of obtained culture mediums, it prepared genome DNA by the method of Saito and a Miura from this microbial cell.

【００２３】
実施例３（Ｎ１３１ａセルラーゼ遺伝子断片のクローニング）
バチルス　エスピー　ＫＳＭ－Ｎ１３１株の培養上清から精製したセルラーゼのアミノ末端配列を１５番目まで決定した結果、Ｇｌｕ－Ｇｌｙ－Ａｓｎ－Ｔｈｒ－Ａｒｇ－Ｇｌｕ－Ａｓｐ－Ａｓｎ－Ｐｈｅ－Ａｓｐ－Ｈｉｓ－Ｌｅｕ－Ｌｅｕ－Ｇｌｙ－Ａｓｎであった。この配列は、バチルス　エスピー　ＫＳＭ－Ｓ２３７株やバチルス　エスピー　ＫＳＭ－６４株の生産するアルカリセルラーゼのアミ

[0023]
Example 3 (cloning of an N131a cellulase gene fragment)
Bacillus sp　It decided the amino-terminus sequence of the cellulase purified from the culture supernatant of 131 strain of KSM-N to the 15th.
As a result, it was Glu-Gly-Asn-Thr-Arg-Glu-Asp-Asn-Phe-Asp-His-Leu-Leu-Gly-Asn.
This sequence is Bacillus sp.　237 strain of KSM-S, and Bacillus sp　Amino-terminus sequence
Glu-Gly-Asn-Thr-Arg-Glu-Asp-Asn-Phe-Lys-His-Leu-Leu-Gly-Asn of the KSM-64 strain alkali cellulase to produce and extremely high

ノ末端配列Ｇｌｕ－Ｇｌｙ－Ａｓｎ－Ｔｈｒ－Ａｒｇ－Ｇｌｕ－Ａｓｐ－Ａｓｎ－Ｐｈｅ－Ｌｙｓ－Ｈｉｓ－ Ｌｅｕ－Ｌｅｕ－Ｇｌｙ－Ａｓｎと極めて高い相同性を示した。そこで中間のアミノ酸配列も相同性が高い可能性があると予想し、Ｓ２３７セルラーゼのアミノ末端及び中間アミノ酸配列を基にプライマー１（配列番号５）及びプライマー２（配列番号６）を合成し、これらを用いてＮ１３１ａセルラーゼをコードする遺伝子の増幅をＰＣＲ反応により試みた。すなわち、バチルス エスピー ＫＳＭ－Ｎ１３１株ゲノム溶液１μＬ（１００ｎｇ）、プライマー１及び２各２０μＬ（１μＭ）、ＰＣＲ用緩衝液１０μＬ、２．５ｍＭｄＮＴＰミックス８μＬ、Ｐｙｒｏｂｅｓｔ ＤＮＡポリメラーゼ（タカラ社製）０．５μＬ（２．５単位）、及び脱イオン水４０μＬを混合し、サーマルサイクラー４８０（パーキンエルマー社製）にて９４℃、２分間の熱変性後、９４℃で１分間、５５℃で１分間、７２℃で２分間を１サイクルとし、３０サイクルの反応条件でＤＮＡの増幅を行った。得られたＰＣＲ産物（約１ｋｂ）をＧＦＸ ＰＣＲ ＤＮＡ ａｎｄ Ｇｅｌ Ｂａｎｄ Ｐｕｒｉｆｉｃａｔｉｏｎ Ｋｉｔ（ファ

homology were shown.

Then, it also anticipates a middle amino acid sequence that homology may be high, it compounds primer 1 (sequence number 5) and primer 2 (sequence number 6) based on the amino terminus and middle amino acid sequence of S237 cellulase, it tried amplification of the gene which codes N131a cellulase using these according to PCR reaction.

Namely, Bacillus sp It mixes 131 strain of KSM-N genome solution 1 micronL (100 ng), primer 1 and 220 micronL each (1 micronM), buffer 10 micronL for PCR, 2.5 mMdNTP mix 8 micronL, PyrobestDNA polymerase (made by Takara company) 0.5 micronL (2.5 unit), and deionized-water 40 micronL, after 94 degrees C and the thermal denaturation for 2 minutes, by 94 degrees C, it makes for 1 minute at 55 degrees C for 1 minute, and makes for 2 minutes into 1 cycle at 72 degrees C at thermal cycler 480 (made by Perkin-Elmer corporation), it performed amplification of DNA on 30-cycle reaction conditions.

GFX PCR DNA and Gel Band Purification Kit (made by Pharmacia K.K.) purifies the acquired PCR production (about 1 kb), it decided the base sequence of the obtained DNA fragment using DNA Sequencing Kit (made by an applied bio-system company), and a 377DNA sequencer (made by a Perkin-Elmer bio-system company).

ルマシア社製）により精製し、得られたＤＮＡ断片の塩基配列をＤＮＡ　Ｓｅｑｕｅｎｃｉｎｇ　Ｋｉｔ（アプライドバイオシステム社製）及び３７７ＤＮＡシークエンサー（パーキンエルマーバイオシステム社製）を用いて決定した。

【００２４】
実施例４（Ｎ１３１ａセルラーゼ遺伝子のゲノムＰＣＲ法によるクローニング）
実施例３で決定したＮ１３１ａセルラーゼ遺伝子は不完全なものであったため、インバースＰＣＲ法により全遺伝子の取得を試みた。すなわち、バチルス　エスピー　ＫＳＭ－Ｎ１３１株ゲノム溶液１０μＬ（８μｇ）、ＰＣＲ用緩衝液５μＬ、脱イオン水３４μＬ及びＥｃｏＲＩ１μＬ（１０単位）を混合し、３７℃、２時間３０分間制限酵素処理した。得られたゲノム分解産物を精製後、Ｌｉｇａｔｉｏｎ　Ｋｉｔ　Ｖｅｒ．２（タカラ社製）を用いて自己閉環した（１６℃、２時間）。自己閉環したＤＮＡを精製し、インバースＰＣＲ法の鋳型として用いた。ＰＣＲ反応は、自己閉環溶液１μＬ、プライマー３（配列番号７）及びプライマー４（配列番号８）各２０μＬ（１μＭ）、ＰＣＲ用緩衝液１０μＬ、２．５ｍＭｄＮＴ

[0024]
Example 4 (cloning by the genome PCR method of an N131a cellulase gene)
Since the N131a cellulase gene decided in Example 3 was imperfect, it tried acquisition of all genes by Inverse PCR method.
Namely, Bacillus sp　It mixes 131 strain of KSM-N genome solution 10 micronL (8 microgram), buffer 5 micronL for PCR, deionized-water 34 micronL, and EcoRI1 micronL (10 unit), 37 degrees C carried out restriction enzyme treatment for 2 hours and 30 minutes.
It carried out the self-ring closure after purifying the acquired genome cleavage product using Ligation Kit Ver.2 (made by the Takara company) (16 degrees C, 2 hours).
It purifies DNA which carried out the self-ring closure, it used as a casting mould of Inverse PCR method.
PCR reaction is self-ring-closure solution 1 micronL, primer 3 (sequence number 7), and primer 4(sequence number 8) 20 micronL each (1 micronM), buffer 10 micronL for PCR, 2.5 mMdNTP mix 8 micronL, after mixing Pyrobest DNA-polymerase 0.5 micronL (2.5 unit) and deionized-water 40.5 micronL, after 94 degrees

Ｐミックス８μＬ、Ｐｙｒｏｂｅｓｔ　ＤＮＡポリメラーゼ０．５μＬ（２．５単位）、及び脱イオン水４０．５μＬを混合した後、９４℃、２分間の熱変性後、９４℃で１分間、５５℃で１分間、７２℃で３分間を１サイクルとし、３０サイクル行った。増幅したＤＮＡ断片（約４ｋｂ）を精製し、このうち約２ｋｂの塩基配列を決定した。この段階で完全なＮ１３１ａセルラーゼ遺伝子及びその上流約５００ｂの配列は決定されたので、次に構造遺伝子下流の塩基配列決定を進め、下流約２００ｂの塩基配列を決定した。得られた塩基配列からセルラーゼ遺伝子の上流領域並びに下流領域の塩基配列を基に、プライマー５（配列番号９）及びプライマー６（配列番号１０）を合成し、バチルス　エスピー　Ｎ１３１株のゲノムからＰＣＲ法によりＮ１３１ａセルラーゼ遺伝子を増幅した。得られた遺伝子の塩基配列を決定し、アミノ酸配列を推定した（配列番号１及び３）。

C and the thermal denaturation for 2 minutes, by 94 degrees C, it makes for 1 minute at 55 degrees C for 1 minute, and makes for 3 minutes into 1 cycle at 72 degrees C, it performed 30 cycles.
It purifies the amplified DNA fragment (about 4 kb(s)), among these, it decided the base sequence of about 2 kb(s).
Since, the sequence of a perfect N131a cellulase gene and its upper approximately 500b was decided in this phase, next, it advanced the base-sequence decision of a structural-gene downstream, and decided the base sequence of down-stream approximately 200b.
Based on the base sequence of the upstream region of a cellulase gene, and a downstream region, it compounds primer 5 (sequence number 9) and primer 6 (sequence number 10) from the obtained base sequence, bacillus sp It amplified the N131a cellulase gene by PCR method from the N131 strain genome.
It decides the base sequence of the obtained gene, it presumed the amino acid sequence (sequence number 1 and 3).

【００２５】
実施例５（形質転換枯草菌によるＮ１３１ａセルラーゼの生産）
Ｎ１３１ａセルラーゼのアミノ末端側からターミネーター下流

[0025]
Example 5 (production of the N131a cellulase by the transforming Bacillus subtilis)
It connects the gene from the amino-terminus side of N131a cellulase to a terminator downstream with the SalI/BamHI part of a

までの遺伝子をプラスミド（ｐＨＳＰ６４）のＳａｌＩ／ＢａｍＨＩ部位に連結し、構築した組換えプラスミドを枯草菌ＩＳＷ１２１４株に導入して形質転換した。形質転換株を３．０重量％ポリペプトンＳ、３．０重量％マルトース、０．５重量％魚肉エキス、０．１重量％酵母エキス、０．１重量％リン酸２水素カリウム、０．０２重量％硫酸マグネシウム７水塩及びテトラサイクリン（７．５μｇ／ｍＬ）から成る培地（ＰＭ培地、ｐＨ６．８）にて３０℃、４８時間振盪培養を行った。遠心分離（８０００×ｇ、２０分間、４℃）により得られた培養上清中のセルラーゼの活性は、約２００００Ｕ／Ｌであった。

plasmid (pHSP64), it introduced the built recombinant plasmid into 1214 strain of Bacillus subtilis ISW, and transformed it.
It performed 30 degrees C and a 48-hour shaking culture in the medium (PM medium, pH6.8) which constitutes the transformant of the 3.0-weight% polypeptone S, the 3.0-weight% maltose, the 0.5-weight% fish-meat extract, the 0.1-weight% yeast extract, 0.1-weight% monobasic potassium phosphate, 0.02-weight% magnesium-sulfate heptahydride, and tetracycline (7.5 microgram/mL).
The activity of the cellulase in the culture supernatant obtained by the centrifugation (for 8000*g and 20 minutes, 4 degrees C) was about 20000 U/L.

【００２６】
実施例６（Ｎ１３１ｂセルラーゼ遺伝子のゲノムＰＣＲ法によるクローニング）
Ｎ１３１ａセルラーゼのクローニングを行った際に、Ｎ１３１ａセルラーゼの配列と類似した配列がバチルス　エスピー　ＫＳＭ－Ｎ１３１株のゲノム上に存在する可能性が示唆された。そこで、Ｎ１３１ａセルラーゼ遺伝子のクローニングの際に用いた方法と同様に、Ｓ２３７セルラーゼのアミノ末端及び中間アミノ酸配列を基にプライマー

[0026]
Example 6 (cloning by the genome PCR method of an N131b cellulase gene)
The sequence which was similar with the sequence of N131a cellulase when a cloning of N131a cellulase was performed is Bacillus sp. Possibility of existing on the genome of 131 strain of KSM-N was suggested.
Then, it compounds a primer 7-12 (sequence number 11-16) based on the amino terminus and middle amino acid sequence of S237 cellulase like the method used on the occasion of a cloning of an N131a cellulase gene, it performed amplification of the gene which codes N131b cellulase by PCR method.

７～１２(配列番号１１～１６)を合成し、ＰＣＲ法によりＮ１３１ｂセルラーゼをコードする遺伝子の増幅を行った。すなわち、バチルス　エスピー　ＫＳＭ－Ｎ１３１株のゲノム溶液１μＬ(７０ｎｇ)、プライマーの組合せを各１０μＬ(０．３μＭ)、ＰＣＲ用緩衝液１０μＬ、２．５ｍＭｄＮＴＰミックス８μＬ、脱イオン水６０μＬ及びＰｗｏＤＮＡポリメラーゼ(ベーリンガーマンハイム社製)１μＬ(５単位)を混合し、９４℃、２分間の熱変性後、９４℃で１分間、５５℃で１分間、７２℃で３分間を１サイクルとし、３０サイクルの反応条件でＤＮＡの増幅を行った。得られたＰＣＲ産物をＨｉｇｈ　Ｐｕｒｅ　ＰＣＲ　Ｐｒｏｄｕｃｔ　Ｐｕｒｉｆｉｃａｔｉｏｎ　Ｋｉｔ(ベーリンガーマンハイム社製)を用いて精製し、３７７ＤＮＡシークンサーにより塩基配列をそれぞれ決定した。得られた遺伝子断片の塩基配列をＳ２３７セルラーゼ遺伝子と比較すると、Ｎ１３１ｂセルラーゼのアミノ末端以降をコードすると考えらるいくつかの遺伝子断片及び停止コドンとその下流域と考えられる遺伝子断片の存在が示唆された。しかし、完全な塩基配列は決定されていないこと並びに開始コドン及びその近傍の

Namely, Bacillus sp　It mixes ten micronL(s) each (0.3 micronM), buffer 10 micronL for PCR, 2.5 mMdNTP mix 8 micronL, deionized-water 60 micronL, and PwoDNA polymerase (made by Boehringer-Mannheim company) 1 micronL (5 unit) for the combination of genome solution 1 micronL (70 ng) of 131 strain of KSM-N, and a primer, after 94 degrees C and the thermal denaturation for 2 minutes, by 94 degrees C, it makes for 1 minute at 55 degrees C for 1 minute, and makes for 3 minutes into 1 cycle at 72 degrees C, it performed amplification of DNA on 30-cycle reaction conditions.

It purifies the acquired PCR production using High Pure PCR Product Purification Kit (made by a Boehringer-Mannheim company), 377DNA sequencer- each decided the base sequence.

The presence of the gene fragment considered to be the gene fragment and the stop codon, and its down-stream region of some which are considered to code the amino terminus of N131b cellulase or subsequent ones compared with a S237 cellulase gene in the base sequence of the obtained gene fragment was suggested.

However, the perfect base sequence was not acquired as a gene fragment about not deciding, the initiating codon, and the region of the vicinity.

First, it is Bacillus sp in order to acquire the gene which codes the upstream region from an amino terminus.　Various restriction enzymes (Sau 3A, EcoRI, HindIII) degrade genome-DNA 4 microgram of 131 strain of KSM-N, it made what was connected with the cassette using the LA PCR in vitro cloning kit

領域については遺伝子断片として取得されていなかった。まず、アミノ末端より上流の領域をコードする遺伝子を取得するためにバチルス　エスピー　ＫＳＭ－Ｎ１３１株のゲノムＤＮＡ４μｇを各種制限酵素（Ｓａｕ３Ａ、ＥｃｏＲＩ、ＨｉｎｄIII）により分解し、ＬＡ　ＰＣＲインヴィトロクローニングキット(宝酒造)を用いてカセットと連結したものを鋳型にＰＣＲ反応［プライマー１３（配列番号１７）及びプライマー１４（配列番号１８）を使用］を行った。その結果、ＨｉｎｄIIIにより処理したサンプルについてＤＮＡの増幅が認められ、この増幅断片（約０．４ｋｂ）の塩基配列を決定した結果、Ｎ１３１ｂセルラーゼのアミノ末端より上流の領域をコードする遺伝子断片が確認された。しかし、その解析を行うと開始コドンから３４塩基下流にアンバーコドン（ＴＧＡ）が存在することが明らかになった。アンバーコドン（ＴＧＡ）に関しては、枯草菌において極くまれにトリプトファンをコードするという報告もあることから（Lovett ら、J. Bacteriol.，173，1810-1812，1991)、本遺伝子においてもトリプトファンをコードする可能性が示唆された。しかし、開始コドンの上流部にはリボソーム

(Takara Shuzo) for the PCR reaction [primer 13 (sequence number 17) and primer 14 (sequence number 18) to the casting mould.

As a result, amplification of DNA is observed about the sample treated by HindIII, it decided the base sequence of this amplification fragment (about 0.4 kb(s)).

As a result, the gene fragment which codes the upstream region from the amino terminus of N131b cellulase was checked.

However, when the analysis was conducted, it became clear from the initiating codon that an amber codon (TGA) exists in 34 base downstream.

Since there is a report of coding the tryptophan rarely extremely in the Bacillus subtilis about an amber codon (TGA) (Lovett et al., J.Bacteriol., 173, 1810-1812, 1991), possibility of coding the tryptophan was also suggested in this gene.

However, a sequence required for the translation start of a ribosome binding site etc. was not discovered by the upper part of the initiating codon, but it also became clear that many ochre codons (TAA) exist.

Therefore, it was thought that this gene had high possibility of being the false gene which does not express in the cell.

In order to decide the base sequence which codes a perfect N131b cellulase gene, it used primer 15 (sequence number 19) and primer 16 (sequence number 20), and performed PCR reaction.

The base sequence eventually decided and the amino acid sequence presumed were shown in sequence number 2 and sequence number 4.

結合部位などの翻訳開始に必要な配列が見出されず、オーカーコドン（ＴＡＡ）がいくつも存在することも明らかになった。従って、本遺伝子は細胞内で発現しない擬似遺伝子である可能性が高いと考えられた。完全なＮ１３１ｂセルラーゼ遺伝子をコードする塩基配列を決定するためにプライマー１５（配列番号１９）及びプライマー１６（配列番号２０）を用いてＰＣＲ反応を行った。最終的に決定された塩基配列及び推定されるアミノ酸配列を配列番号２及び配列番号４に示した。

【００２７】
実施例７（形質転換枯草菌によるＮ１３１ｂセルラーゼの生産）
細胞内で発現しない可能性のあるＮ１３１ｂセルラーゼを生産させる目的で、遺伝子の発現に必要な領域としてバチルス　エスピー　ＫＳＭ－６４株由来のアルカリセルラーゼ遺伝子（Sumitomo　ら　、　Biosci. Biotechnol. Biochem.，56，827-877，1992）の上流発現領域を増幅した［プライマー１７（配列番号２１）及びプライマー１８（配列番号２２）を使用］。得られたＮ１３１ｂセルラーゼ遺伝子断片と上流発現領域遺伝子断片を精製し、プライマー１

[0027]
Example 7 (production of the N131b cellulase by the transforming Bacillus subtilis)
It is Bacillus sp as region required for the expression of a gene in order to produce the N131b cellulase which may not express in the cell.　It is the [primer 17 (sequence number 21) and primer 18 (sequence number 22) which amplified the upper expression region of the alkali cellulase gene (Biosci. Biotechnol. Sumitomo et al., Biochem., 56,827- 877, 1992) derived from KSM-64 strain Use]
It purifies the N131b cellulase gene fragment and the upper expression region gene fragment which were obtained, recombinant PCR method performed amplification of DNA using primer 16 (sequence number 20) and primer 17 (sequence number 21).
It purifies the acquired chimera gene, it

６（配列番号２０）及びプライマー１７（配列番号２１）を用いてリコンビナントＰＣＲ法によりＤＮＡの増幅を行った。取得したキメラ遺伝子を精製し、制限酵素Ｂｇｌ II びＨｉｎｄIII で処理後、予め同じ制限酵素で処理しておいたプラスミドｐＨＹ３００ＰＬＫ（ヤクルト本社製）に連結した。得られた組換えプラスミドをプロトプラスト法により枯草菌ＩＳＷ１２１４株に導入し、形質転換を行った。形質転換株をＰＭ培地（テトラサイクリンは１５μｇ／ｍＬとした）中で３０℃、７２時間振盪培養した。遠心分離により得られた培養上清中のセルラーゼの活性は、約３３０００Ｕ／Ｌであった。

connected with plasmid pHY300PLK (made by Yakult Honsha) treated beforehand at the same restriction enzyme after treatment by restriction enzyme BglII HindIII.
It introduces the obtained recombinant plasmid into 1214 strain of Bacillus subtilis ISW by the protoplast method, it performed transforming.
It carried out the shaking culture of the 30 degrees C of the transformant for 72 hours in PM medium (it set the tetracycline to 15 microgram(s)/mL).
The activity of the cellulase in the culture supernatant obtained by the centrifugation was about 33000 U/L.

【００２８】
［酵素活性測定法］０．２ｍＬの０．５Ｍグリシン－水酸化ナトリウム緩衝液（ｐＨ９．０）、０．４ｍＬの２．５重量％カルボキシメチルセルロース（Ａ０１ＭＣ；日本製紙社製）及び０．３ｍＬの脱イオン水から成る反応液に、適当に希釈した０．１ｍＬの酵素液を加えて２０分間反応させた後、１ｍＬのジニトロサリチル酸試薬（０．５重量％ジニトロサリチル酸、３０重量％ロッシェル塩、１．６重量％水酸化ナトリウム水溶液）を添

[0028]
[Enzyme active measuring method]
0.2 mL 0.5M glycine- sodium-hydroxide buffer (pH9.0), 0.4 mL 2.5-weight% carboxymethylcellulose (A01MC;)
After adding the 0.1 mL enzyme liquid diluted suitably to the reaction mixture which constitutes of the Nippon Paper Industries make and a 0.3 mL deionized water and letting it react to it for 20 minutes, it adds the 1 mL dinitro salicylic-acid reagent (the 0.5-weight% dinitro salicylic acid, the 30-weight% Rochelle salt, the 1.6-weight% sodium-hydroxide aqueous solution), it performed the color development of the reducing sugar for 5 minutes in boiling

加し、沸水中で５分間還元糖の発色を行った。氷水中で急冷し、４ｍＬの脱イオン水を加え、５３５ｎｍにおける吸光度を測定して還元糖の生成量を求めた。尚、ブランクは酵素液を加えずに処理した反応液にジニトロサリチル酸試薬を加えた後、酵素液を添加し、同様に発色させたものを用意した。酵素１単位（１Ｕ）は、上記反応条件下において１分間に１μｍｏｌのグルコース相当の還元糖を生成する量とした。

water.
It quenches in ice water, it added the 4 mL deionized water, it measured the absorbence in 535 nm, and calculated the produced amount of the reducing sugar.
In addition, a blank adds enzyme liquid, after adding the dinitro salicylic-acid reagent to the reaction mixture treated without adding enzyme liquid, it prepared what was developed colors similarly.
It made 1 unit (1U) of enzymes into the quantity which forms the reducing sugar of the glucose of 1 micrometerol in 1 minute on the above-mentioned reaction conditions.

【００２９】
参考例１（Ｎ１３１ａセルラーゼの最適反応ｐＨ）
クエン酸緩衝液（ｐＨ４－７）、リン酸緩衝液（ｐＨ６－８）、トリス－塩酸緩衝液（ｐＨ７－９）、グリシン－水酸化ナトリウム緩衝液（ｐＨ８－１１）、リン酸－水酸化ナトリウム緩衝液（ｐＨ１２－１２．５）の各緩衝液（１００ｍＭ）を用いて最適反応ｐＨを調べた結果、Ｎ１３１ａセルラーゼはｐＨ９－９．５のグリシン－水酸化ナトリウム緩衝液中で最も高い反応速度を示した。また、ｐＨ７から１１の間で最大活性の５０％以上の活性を有していた（図１）。

[0029]
Reference Example 1 (the optimal reaction pH of N131a cellulase)
It examined the optimal reaction pH using each buffer (100 mM) of citrate buffer solution (pH4-7), a phosphate buffer (pH6-8), tris-hydrochloric-acid buffer (pH7-9), glycine-sodium-hydroxide buffer (pH8-11), and phosphoric-acid-sodium-hydroxide buffer (pH12-12.5).
As a result, N131a cellulase showed the highest reaction rate in the glycine- sodium-hydroxide buffer of pH9-9.5.
Moreover, it had the activity of 50 % or more of the maximum activity between 11 from pH7 (FIG. 1).

【００３０】

[0030]

参考例２（Ｎ１３１ｂセルラーゼの最適反応ｐＨ）
参考例１と同様にしてＮ１３１ｂセルラーゼの最適反応ｐＨを調べた結果、ｐＨ９－９．５のグリシン－水酸化ナトリウム緩衝液中で最も高い反応速度を示した。また、ｐＨ７から１１の間で最大活性の５０％以上の活性を有していた（図２）。

Reference Example 2 (the optimal reaction pH of N131b cellulase)
It examined the optimal reaction pH of N131b cellulase like Reference Example 1.
As a result, the highest reaction rate in the glycine- sodium-hydroxide buffer of pH9-9.5 was shown.
Moreover, it had the activity of 50 % or more of the maximum activity between 11 from pH7 (FIG. 2).

【００３１】

[0031]

【発明の効果】
本発明のアルカリセルラーゼ遺伝子を用いれば、衣料用洗剤、繊維処理剤等として有用なアルカリセルラーゼを単一且つ大量に生産することが可能である。

[ADVANTAGE OF THE INVENTION]
If the alkali cellulase gene of this invention is used, alkali cellulase useful as the detergent for garments, a fiber processing agent, etc. is producible individually and in large quantities.

【００３２】

[0032]

【配列表】
SEQUENCE LISTING
<110> KAO CORPORATION
<120> Gene for Alkaline Cellulase

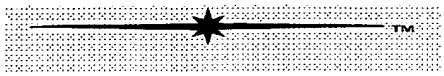
<130> P00741202
<160> 22
<210> 1
<211> 859

<212> PRT
<213> Bacillus sp.
<400> 1

[SEQUENCE TABLE]
SEQUENCE LISTING
<110> KAO CORPORATION
<120> Gene for Alkaline Cellulase

<130> P00741202
<160> 22
<210> 1
<211> 859

<212> PRT
<213> Bacillus sp.
<400> 1



Met Met Leu Arg Lys Lys Thr Lys Gln Leu Ile Ser Ser Thr Leu Ile

5 10 15

Leu Val Leu Leu Leu Ser Leu Phe Pro Thr Ala Leu Ala Ala Glu Gly

20 25 30

Asn Thr Arg Glu Asp Asn Phe Asp His Leu Leu Gly Asn Glu Asn Val

35 40 45

Lys Arg Pro Ser Glu Ala Gly Ala Leu Gln Leu Lys Glu Val Asp Gly

50 55 60

Gln Met Thr Leu Val Asp Gln His Gly Glu Lys Ile Gln Leu Arg Gly

65 70 75 80

Met Ser Thr His Gly Leu Gln Trp Phe Pro Glu Ile Leu Asn Asp Asn

85 90 95

Ala Tyr Lys Ala Leu Ser Asn Asp Trp Asp Ser Asn Met Ile Arg Leu

Met Met Leu Arg Lys Lys Thr Lys Gln Leu Ile Ser Ser Thr Leu Ile

5 10 15

Leu Val Leu Leu Leu Ser Leu Phe Pro Thr Ala Leu Ala Ala Glu Gly

20 25 30

Asn Thr Arg Glu Asp Asn Phe Asp His Leu Leu Gly Asn Glu Asn Val

35 40 45

Lys Arg Pro Ser Glu Ala Gly Ala Leu Gln Leu Lys Glu Val Asp Gly

50 55 60

Gln Met Thr Leu Val Asp Gln His Gly Glu Lys Ile Gln Leu Arg Gly

65 70 75 80

Met Ser Thr His Gly Leu Gln Trp Phe Pro Glu Ile Leu Asn Asp Asn

85 90 95

Ala Tyr Lys Ala Leu Ser Asn Asp Trp Asp Ser Asn Met Ile Arg Leu

100 105 110
Ala Met Tyr Val Gly Glu Asn Gly Tyr Ala Thr Asn Pro Glu Leu Ile
115 120 125
Lys Gln Arg Val Ile Asp Gly Ile Glu Leu Ala Ile Glu Asn Asp Met

130 135 140
Tyr Val Ile Val Asp Trp His Val His Ala Pro Gly Asp Pro Arg Asp
145 150 155 160
Pro Val Tyr Ala Gly Ala Glu Asp Phe Phe Arg Asp Ile Ala Ala Leu

165 170 175
Tyr Pro Asn Asn Arg His Ile Ile Tyr Glu Leu Ala Asn Glu Pro Ser
180 185 190
Ser Asn Asn Asn Gly Gly Ala Gly Ile Pro Asn Asn Glu Glu Gly Trp

195 200 205
Lys Ala Val Lys Glu Tyr Ala Asp

100 105 110
Ala Met Tyr Val Gly Glu Asn Gly Tyr Ala Thr Asn Pro Glu Leu Ile
115 120 125
Lys Gln Arg Val Ile Asp Gly Ile Glu Leu Ala Ile Glu Asn Asp Met

130 135 140
Tyr Val Ile Val Asp Trp His Val His Ala Pro Gly Asp Pro Arg Asp
145 150 155 160
Pro Val Tyr Ala Gly Ala Glu Asp Phe Phe Arg Asp Ile Ala Ala Leu

165 170 175
Tyr Pro Asn Asn Arg His Ile Ile Tyr Glu Leu Ala Asn Glu Pro Ser
180 185 190
Ser Asn Asn Asn Gly Gly Ala Gly Ile Pro Asn Asn Glu Glu Gly Trp

195 200 205
Lys Ala Val Lys Glu Tyr Ala Asp Pro Ile Val Glu

Pro Ile Val Glu Met Leu Arg Asp
210 215
220
Ser Gly Asn Ala Asp Asp Asn
Ile Ile Ile Val Gly Ser Pro Asn
Trp

225 230
235 240
Ser Gln Arg Pro Asp Leu Ala
Ala Asp Asn Pro Ile Asn Asp His
His
245
250 255
Thr Met Tyr Thr Val His Phe Tyr
Ser Gly Ser His Ala Ala Ser Thr

260
265 270
Glu Ser Tyr Pro Pro Glu Thr Pro
Asn Ser Glu Arg Gly Asn Val
Met
275
280 285
Ser Asn Thr Arg Tyr Ala Leu
Glu Asn Gly Val Ala Val Phe Ala
Thr

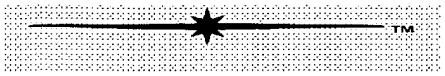
290 295
300
Glu Trp Gly Thr Ser Gln Ala Asn
Gly Asp Gly Gly Pro Tyr Phe
Asp
305 310
315 320
Glu Ala Asp Val Trp Ile Glu Phe

Met Leu Arg Asp
210 215
220
Ser Gly Asn Ala Asp Asp Asn Ile Ile Ile Val Gly
Ser Pro Asn Trp

225 230
235 240
Ser Gln Arg Pro Asp Leu Ala Ala Asp Asn Pro
Ile Asn Asp His His
245 250
255
Thr Met Tyr Thr Val His Phe Tyr Ser Gly Ser His
Ala Ala Ser Thr

260 265
270
Glu Ser Tyr Pro Pro Glu Thr Pro Asn Ser Glu
Arg Gly Asn Val Met
275 280
285
Ser Asn Thr Arg Tyr Ala Leu Glu Asn Gly Val Ala
Val Phe Ala Thr

290 295
300
Glu Trp Gly Thr Ser Gln Ala Asn Gly Asp Gly
Gly Pro Tyr Phe Asp
305 310
315 320
Glu Ala Asp Val Trp Ile Glu Phe Leu Asn Glu
Asn Asn Ile Ser Trp



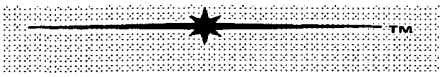
Leu Asn Glu Asn Asn Ile Ser
Trp

325 325 330
330 335 335
Ala Asn Trp Ser Leu Thr Asn Ala Asn Trp Ser Leu Thr Asn Lys Asn Glu Val
Lys Asn Glu Val Ser Gly Ala Ser Gly Ala Phe Thr
Phe Thr 340 345
340 350
345 350 Pro Phe Glu Leu Gly Lys Ser Asn Ala Thr Ser
Pro Phe Glu Leu Gly Lys Ser Leu Asp Pro Gly Pro
Asn Ala Thr Ser Leu Asp Pro
Gly Pro

355 355 360
360 365 365
Asp Gln Val Trp Ala Pro Glu Glu Asp Gln Val Trp Ala Pro Glu Glu Leu Ser Leu
Leu Ser Leu Ser Gly Glu Tyr Val Ser Gly Glu Tyr Val
370 375 370 375
380 380
Arg Ala Arg Ile Lys Gly Ala Lys Arg Ala Arg Ile Lys Gly Ala Lys Tyr Glu Pro Ile
Tyr Glu Pro Ile Asp Arg Thr Arg Asp Arg Thr Arg

385 390 385 390
395 400 395 400
Tyr Thr Lys Val Leu Trp Asp Tyr Thr Lys Val Leu Trp Asp Phe Asn Asp Gly
Phe Asn Asp Gly Thr Lys Gln Thr Lys Gln Gly Phe
Gly Phe 405 410
405 415
410 415 Gly Val Asn Ser Asp Ser Pro Asn Lys Glu Ala Ile
Gly Val Asn Ser Asp Ser Pro Glu Val Glu Asn
Asn Lys Glu Ala Ile Glu Val Glu
Asn

420 420 425
425 430 430



Glu Asn Gly Thr Leu Arg Ile Ser Gly Leu Asn Val Ser Asn Asp Leu
435 440 445
Ser Asp Gly Asn Phe Trp Ala Asn Val Arg Leu Ser Ala Asn Gly Trp
450 455 460
Gly Lys Ser Val Asp Ile Leu Ser Ala Glu Lys Leu Thr Met Asp Gly
465 470 475 480
Ile Val Asp Glu Pro Thr Thr Val Ala Ile Ala Ala Ile Pro Gln Ser
485 490 495
Thr Lys His Gly Trp Ala Asn Pro Glu Arg Ser Val Lys Val Thr Glu
500 505 510
Ala Asp Phe Val Lys Gln Asp Asp Gly Lys Tyr Lys Ala Leu Leu Thr
515 520 525
Ile Thr Gly Asp Asp Ala Pro Asn Leu Lys Asn Ile Gly Phe Asp Asp
530 535 540

Glu Asn Gly Thr Leu Arg Ile Ser Gly Leu Asn Val Ser Asn Asp Leu
435 440 445
Ser Asp Gly Asn Phe Trp Ala Asn Val Arg Leu Ser Ala Asn Gly Trp
450 455 460
Gly Lys Ser Val Asp Ile Leu Ser Ala Glu Lys Leu Thr Met Asp Gly
465 470 475 480
Ile Val Asp Glu Pro Thr Thr Val Ala Ile Ala Ala Ile Pro Gln Ser
485 490 495
Thr Lys His Gly Trp Ala Asn Pro Glu Arg Ser Val Lys Val Thr Glu
500 505 510
Ala Asp Phe Val Lys Gln Asp Asp Gly Lys Tyr Lys Ala Leu Leu Thr
515 520 525
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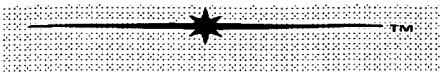
530 535 540
Glu Asn Asn Asn Met Asn Asn Ile Ile Leu Phe



Glu Asn Asn Asn Met Asn Asn Ile Ile Leu Phe Val Gly Thr Glu Ala

Val Gly Thr Glu Ala

545 550 555 560

Ala Asp Val Ile Tyr Leu Asp Asn Ile Lys Val Thr Gly Lys Ile Val

565 570 575

Glu Ile Pro Val Val His Ser Pro Lys Gly Asp Ala Ala Leu Pro Ser

580 585 590

Asn Phe Glu Asp Gly Thr Arg Gln Gly Trp Asp Trp Ala Gly Glu Ser

595 600 605

Gly Val Lys Thr Ala Leu Thr Ile Glu Glu Ala Asn Gly Ser Gln Ala

610 615 620

Leu Ser Trp Glu Phe Gly Tyr Pro Glu Val Lys Pro Ser Asp Asn Trp

625 630 635 640

Ala Ser Ala Pro Arg Leu Asp Phe His Lys Asp Asn Leu Val Arg Gly

645 650

545 550 555 560

Ala Asp Val Ile Tyr Leu Asp Asn Ile Lys Val Thr Gly Lys Ile Val

565 570 575

Glu Ile Pro Val Val His Ser Pro Lys Gly Asp Ala Ala Leu Pro Ser

580 585 590

Asn Phe Glu Asp Gly Thr Arg Gln Gly Trp Asp Trp Ala Gly Glu Ser

595 600 605

Gly Val Lys Thr Ala Leu Thr Ile Glu Glu Ala Asn Gly Ser Gln Ala

610 615 620

Leu Ser Trp Glu Phe Gly Tyr Pro Glu Val Lys Pro Ser Asp Asn Trp

625 630 635 640

Ala Ser Ala Pro Arg Leu Asp Phe His Lys Asp Asn Leu Val Arg Gly

645 650

650 655
Glu Asn Asp Tyr Val Ala Phe Asp Phe Tyr Ile Asp Pro Ala Arg Ala
660
665 670
Thr Glu Gly Ala Met Asn Ile Asn Leu Val Phe Gln Pro Pro Ala Asn

675
680 685
Gly Tyr Trp Val Gln Ala Pro Lys Thr Phe Thr Ile Asn Phe Glu Glu
690 695
700
Leu Glu Glu Ala Asn Gln Val Asn Gly Leu Tyr His Tyr Glu Val Lys

705 710
715 720
Ile Asn Val Arg Asp Ile Ala Asn Ile Gln Asp Asp Thr Val Leu Arg
725
730 735
Asn Met Ile Leu Ile Phe Ala Asp Val Gln Ser Asp Phe Ala Gly Arg

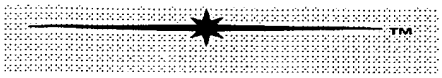
740
745 750
Val Phe Val Asp Asn Val Arg Phe Glu Ala Ser Ala Thr Glu Pro Val

655
Glu Asn Asp Tyr Val Ala Phe Asp Phe Tyr Ile Asp Pro Ala Arg Ala
660 665
670
Thr Glu Gly Ala Met Asn Ile Asn Leu Val Phe Gln Pro Pro Ala Asn

675 680
685
Gly Tyr Trp Val Gln Ala Pro Lys Thr Phe Thr Ile Asn Phe Glu Glu
690 695
700
Leu Glu Glu Ala Asn Gln Val Asn Gly Leu Tyr His Tyr Glu Val Lys

705 710
715 720
Ile Asn Val Arg Asp Ile Ala Asn Ile Gln Asp Asp Thr Val Leu Arg
725 730
735
Asn Met Ile Leu Ile Phe Ala Asp Val Gln Ser Asp Phe Ala Gly Arg

740 745
750
Val Phe Val Asp Asn Val Arg Phe Glu Ala Ser Ala Thr Glu Pro Val
755 760



755 760 765
Glu Pro Val Glu Pro Val Asp Pro Ala Pro Val Glu Pro Glu Pro Val

770 775 780
Asp Pro Gly Glu Glu Thr Pro Pro Val Asp Glu Lys Glu Ala Ala Lys
785 790 795 800
Glu Glu Arg Glu Ala Ala Lys Ala Glu Arg Glu Ala Ala Arg Glu Ala

805 810 815
Ala Lys Glu Glu Arg Glu Glu Ala Arg Glu Ala Ala Lys Glu Glu Arg
820 825 830
Glu Ala Ala Lys Ala Glu Arg Glu Ala Ala Arg Glu Ala Ala Lys Ala

835 840 845
Glu Arg Glu Ala Lys Lys Glu Ala Lys Lys Lys
850 855

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<212> PRT

<213> Bacillus sp.

765
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770 775 780
Asp Pro Gly Glu Glu Thr Pro Pro Val Asp Glu Lys Glu Ala Ala Lys
785 790 795 800
Glu Glu Arg Glu Ala Ala Lys Ala Glu Arg Glu Ala Ala Arg Glu Ala

805 810 815
Ala Lys Glu Glu Arg Glu Glu Ala Arg Glu Ala Ala Lys Glu Glu Arg
820 825 830
Glu Ala Ala Lys Ala Glu Arg Glu Ala Ala Arg Glu Ala Ala Lys Ala

835 840 845
Glu Arg Glu Ala Lys Lys Glu Ala Lys Lys Lys
850 855

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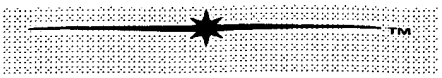

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Leu Ala Ala Glu Gly Asn Thr Arg Glu Asp Asn Phe Lys His Leu Leu
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Gly Asn Asp Asn Val Lys Arg Pro Ser Glu Ala Gly Ala Leu Gln Leu
35 40 45

Gln Glu Val Asp Gly Gln Met Thr Leu Val Asp Gln His Gly Glu Lys
50 55 60

Ile Gln Leu Arg Gly Met Ser Thr His Gly Leu Gln Trp Phe Pro Glu
65 70 75 80

Ile Leu Asn Asp Asn Ala Tyr Lys Ala Leu Ser Asn Asp Trp Asp Ser
85 90 95

Asn Met Ile Arg Leu Ala Met Tyr Val Gly Glu Asn Gly His Ala Thr

<400> 2
Met Leu Leu His Gln Leu Leu Ile Phe Glu Gly(Trp)Ser Gln Lys Val
5 10 15

Leu Ala Ala Glu Gly Asn Thr Arg Glu Asp Asn Phe Lys His Leu Leu
20 25 30

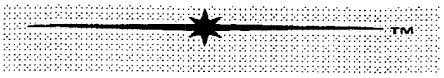
Gly Asn Asp Asn Val Lys Arg Pro Ser Glu Ala Gly Ala Leu Gln Leu
35 40 45

Gln Glu Val Asp Gly Gln Met Thr Leu Val Asp Gln His Gly Glu Lys
50 55 60

Ile Gln Leu Arg Gly Met Ser Thr His Gly Leu Gln Trp Phe Pro Glu
65 70 75 80

Ile Leu Asn Asp Asn Ala Tyr Lys Ala Leu Ser Asn Asp Trp Asp Ser
85 90 95

Asn Met Ile Arg Leu Ala Met Tyr Val Gly Glu Asn Gly His Ala Thr
100 105



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105 110

Asn Pro Glu Leu Ile Lys Gln Arg Val Ile Asp Gly Ile Glu Leu Ala
115 120
125
Ile Glu Asn Asp Met Tyr Val Ile Val Asp Trp His Val His Ala Pro
130 135
140

Gly Asp Pro Arg Asp Pro Val Tyr Ala Gly Ala Lys Asp Phe Phe Arg
145 150
155 160
Glu Ile Ala Ala Leu Tyr Pro Asn Asn Pro His Ile Ile Tyr Glu Leu
165
170 175

Ala Asn Glu Pro Ser Ser Asn Asn Asn Gly Gly Ala Gly Ile Pro Asn
180
185 190
Asn Glu Glu Gly Trp Lys Ala Val Lys Glu Tyr Ala Asp Pro Ile Val
195
200 205

Gln Met Leu Arg Lys Ser Gly Asn Ala Asp Asp Asn Ile Ile Ile Val
210 215

Asn Pro Glu Leu Ile Lys Gln Arg Val Ile Asp Gly Ile Glu Leu Ala
115 120
125
Ile Glu Asn Asp Met Tyr Val Ile Val Asp Trp His Val His Ala Pro
130 135
140

Gly Asp Pro Arg Asp Pro Val Tyr Ala Gly Ala Lys Asp Phe Phe Arg
145 150
155 160
Glu Ile Ala Ala Leu Tyr Pro Asn Asn Pro His Ile Ile Tyr Glu Leu
165 170
175

Ala Asn Glu Pro Ser Ser Asn Asn Asn Gly Gly Ala Gly Ile Pro Asn
180 185
190
Asn Glu Glu Gly Trp Lys Ala Val Lys Glu Tyr Ala Asp Pro Ile Val
195 200
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Gln Met Leu Arg Lys Ser Gly Asn Ala Asp Asp Asn Ile Ile Ile Val
210 215
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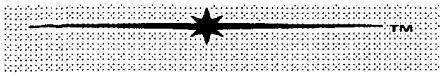

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Gly Ser Pro Asn Trp Ser Gln
Arg Pro Asp Leu Ala Ala Asp
Asn Pro
225 230
235 240

Gly Ser Pro Asn Trp Ser Gln Arg Pro Asp Leu
Ala Ala Asp Asn Pro
225 230
235 240

Ile Asp Asp His His Thr Met Tyr
Thr Val His Phe Tyr Thr Gly Ser
245
250 255
His Ala Ala Ser Thr Glu Ser Tyr
Pro Pro Glu Thr Pro Asn Ser
Glu
260
265 270

Ile Asp Asp His His Thr Met Tyr Thr Val His Phe
Tyr Thr Gly Ser
245 250
255
His Ala Ala Ser Thr Glu Ser Tyr Pro Pro Glu Thr
Pro Asn Ser Glu
260 265
270

Arg Gly Asn Val Met Ser Asn
Thr Arg Tyr Ala Leu Glu Asn Gly
Val
275
280 285
Ala Val Phe Ala Thr Glu Trp Gly
Thr Ser Gln Ala Asn Gly Asp
Gly
290 295
300

Arg Gly Asn Val Met Ser Asn Thr Arg Tyr Ala
Leu Glu Asn Gly Val
275 280
285
Ala Val Phe Ala Thr Glu Trp Gly Thr Ser Gln Ala
Asn Gly Asp Gly
290 295
300

Gly Pro Tyr Phe Asp Glu Ala
Asp Val Trp Ile Glu Phe Leu
Asn Glu
305 310
315 320
Asn Asn Ile Ser Trp Ala Asn Trp
Ser Leu Thr Asn Lys Asn Glu
Val

Gly Pro Tyr Phe Asp Glu Ala Asp Val Trp Ile Glu
Phe Leu Asn Glu
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315 320
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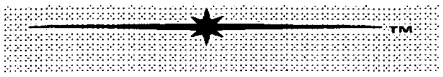
Asn Lys Asn Glu Val
325 330
335



325
330 335

Ser Gly Ala Phe Thr Pro Phe Glu Leu Gly Lys Ser Asn Ala Thr Ser
340 345 350

Leu Asp Pro Gly Pro Asp Gln Val Trp Val Pro Glu Glu Leu Ser Leu
355 360 365

Ser Gly Glu Tyr Val Arg Ala Arg Ile Lys Gly Val Asn Tyr Glu Pro
370 375 380

Ile Asp Arg Thr Lys Tyr Thr Lys Val Leu Trp Asp Phe Asn Asp Gly
385 390 395 400

Thr Lys Gln Gly Phe Gly Val Asn Ser Asp Ser Pro Asn Lys Glu Leu
405 410 415

Ile Ala Val Asp Asn Glu Asn Asn Thr Leu Lys Val Ser Gly Leu Asp
420 425 430

Val Ser Asn Asp Val Ser Asp

Ser Gly Ala Phe Thr Pro Phe Glu Leu Gly Lys Ser Asn Ala Thr Ser
340 345 350

Leu Asp Pro Gly Pro Asp Gln Val Trp Val Pro Glu Glu Leu Ser Leu
355 360 365

Ser Gly Glu Tyr Val Arg Ala Arg Ile Lys Gly Val Asn Tyr Glu Pro
370 375 380

Ile Asp Arg Thr Lys Tyr Thr Lys Val Leu Trp Asp Phe Asn Asp Gly
385 390 395 400

Thr Lys Gln Gly Phe Gly Val Asn Ser Asp Ser Pro Asn Lys Glu Leu
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Ile Ala Val Asp Asn Glu Asn Asn Thr Leu Lys Val Ser Gly Leu Asp
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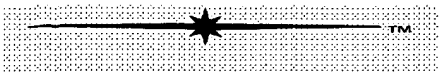
Val Ser Asn Asp Val Ser Asp Gly Asn Phe Trp



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Ser Ala Asn Gly Trp Gly Lys Ser Val Asp Ile Leu Gly Ala Glu Lys
450 455 460

Leu Thr Met Asp Val Ile Val Asp Glu Pro Thr Thr Val Ala Ile Ala
465 470 475 480
Ala Ile Pro Gln Ser Ser Lys Ser Gly Trp Ala Asn Pro Glu Arg Ala
485 490 495

Val Arg Val Asn Ala Glu Asp Phe Val Gln Gln Thr Asp Gly Lys Tyr
500 505 510
Lys Ala Gly Leu Thr Ile Thr Gly Glu Asp Ala Pro Ser Leu Glu Ala
515 520 525

Ile Ala Met His Ala Glu Asn Tyr Thr Ile Asn Asn Ile Ile Leu Phe
530 535 540
Val Gly Thr Glu Gly Ala Asp Val Ile Tyr Leu Asp Thr Ile Lys Val
545 550

Ala Asn Ala Arg Leu
435 440 445
Ser Ala Asn Gly Trp Gly Lys Ser Val Asp Ile Leu Gly Ala Glu Lys
450 455 460

Leu Thr Met Asp Val Ile Val Asp Glu Pro Thr Thr Val Ala Ile Ala
465 470 475 480
Ala Ile Pro Gln Ser Ser Lys Ser Gly Trp Ala Asn Pro Glu Arg Ala
485 490 495

Val Arg Val Asn Ala Glu Asp Phe Val Gln Gln Thr Asp Gly Lys Tyr
500 505 510
Lys Ala Gly Leu Thr Ile Thr Gly Glu Asp Ala Pro Ser Leu Glu Ala
515 520 525

Ile Ala Met His Ala Glu Asn Tyr Thr Ile Asn Asn Ile Ile Leu Phe
530 535 540
Val Gly Thr Glu Gly Ala Asp Val Ile Tyr Leu Asp Thr Ile Lys Val
545 550

555 560

Ile Gly Pro Glu Val Glu Ile Pro Val Val His Asp Pro Lys Gly Glu
565 570 575

Ala Val Leu Pro Ser Val Phe Glu Asp Gly Thr Arg Gln Gly Trp Asp
580 585 590

Trp Ala Gly Glu Ser Gly Val Lys Thr Ala Leu Thr Ile Glu Glu Ala
595 600 605

Asn Gly Ser Asn Ala Leu Ser Trp Glu Phe Gly Tyr Pro Glu Val Lys
610 615 620

Pro Ser Asp Asn Trp Ala Thr Ala Pro Arg Leu Asp Phe Trp Lys Ser
625 630 635 640

Asp Leu Val Arg Gly Glu Asn Asp Tyr Val Thr Phe Asp Phe Tyr Leu
645 650 655

Asp Pro Val Arg Ala Thr Glu Gly Ala Met Asn Ile Asn Leu Val Phe
660 665

555 560

Ile Gly Pro Glu Val Glu Ile Pro Val Val His Asp Pro Lys Gly Glu
565 570 575

Ala Val Leu Pro Ser Val Phe Glu Asp Gly Thr Arg Gln Gly Trp Asp
580 585 590

Trp Ala Gly Glu Ser Gly Val Lys Thr Ala Leu Thr Ile Glu Glu Ala
595 600 605

Asn Gly Ser Asn Ala Leu Ser Trp Glu Phe Gly Tyr Pro Glu Val Lys
610 615 620

Pro Ser Asp Asn Trp Ala Thr Ala Pro Arg Leu Asp Phe Trp Lys Ser
625 630 635 640

Asp Leu Val Arg Gly Glu Asn Asp Tyr Val Thr Phe Asp Phe Tyr Leu
645 650 655

Asp Pro Val Arg Ala Thr Glu Gly Ala Met Asn Ile Asn Leu Val Phe
660 665

660 665 670
Gln Pro Pro Thr Asn Gly Tyr Trp Val Gln Ala Pro Lys Thr Tyr Thr
675 680 685

670 675 680
Gln Pro Pro Thr Asn Gly Tyr Trp Val Gln Ala Pro Lys Thr Tyr Thr
685

Ile Asn Phe Asp Glu Leu Glu Glu Ala Asn Gln Val Asn Gly Leu Tyr
690 695 700
His Tyr Glu Val Lys Ile Asn Val Arg Asp Ile Thr Asn Ile Gln Asp
705 710 715 720

Ile Asn Phe Asp Glu Leu Glu Glu Ala Asn Gln Val Asn Gly Leu Tyr
690 695 700
His Tyr Glu Val Lys Ile Asn Val Arg Asp Ile Thr Asn Ile Gln Asp
705 710 715 720

Asp Thr Leu Leu Arg Asn Met Met Ile Ile Phe Ala Asp Val Glu Ser
725 730 735
Asp Phe Ala Gly Arg Val Phe Val Asp Asn Val Arg Phe Glu Gly Ala
740 745 750

Asp Thr Leu Leu Arg Asn Met Met Ile Ile Phe Ala Asp Val Glu Ser
725 730 735
Asp Phe Ala Gly Arg Val Phe Val Asp Asn Val Arg Phe Glu Gly Ala
740 745 750

Ala Thr Thr Glu Pro Val Glu Pro Glu Pro Val Asp Pro Gly Glu Glu
755 760 765
Thr Pro Pro Val Asp Glu Lys Glu Ala Lys Lys Glu Gln Lys Glu Ala
770 775 780

Ala Thr Thr Glu Pro Val Glu Pro Glu Pro Val Asp Pro Gly Glu Glu
755 760 765
Thr Pro Pro Val Asp Glu Lys Glu Ala Lys Lys Glu Gln Lys Glu Ala
770 775 780

770 775
780

Glu Lys Glu Glu Lys Glu Ala Val Lys Glu Glu Lys Lys Glu Ala Lys
785 790
795 800
Glu Glu Lys Lys Ala Ile Lys Asn Glu Ala Thr Lys Lys
805
810

Glu Lys Glu Glu Lys Glu Ala Val Lys Glu Glu Lys Lys Glu Ala Lys
785 790
795 800
Glu Glu Lys Lys Ala Ile Lys Asn Glu Ala Thr Lys Lys
805 810

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<213> Bacillus sp.
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aaaatactat ttcgaaagcg gtttacacaa aaaaccttat gttatggcgt ttttagataa 180
ttggaagaaa aaaacaactc tagtaatcta aattgaacat gctataatga gtttgttagc 240
agcaatatcg gtgttattta cttactaata atgtaagcgt ttaacctaag agtagacgct 300
tatatccgaa ggaggtagat

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Aaaatactat ttcgaaagcg gtttacacaa aaaaccttat gttatggcgt ttttagataa 180
Ttggaagaaa aaaacaactc tagtaatcta aattgaacat gctataatga gtttgttagc 240
Agcaatatcg gtgttattta cttactaata atgtaagcgt ttaacctaag agtagacgct 300
Tatatccgaa ggaggtagat tgagtcaagt aggaataatg atgatgcagt gtacgagctt 360

tgagtcaagt aggaataatg atgatgcagt gtacgagctt 360

taaagaatca ggagagcact tcgggttact cccgtatttt tttggaatgt tttcgaaagc 420
actttcggta ttttagttat ttgactcaat taagancgat aattaggagg taat atg 477
Met
atg tta aga aag aaa aca aag cag ttg att tct tcc act ctt att tta 525
Met Leu Arg Lys Lys Thr Lys Gln Leu Ile Ser Ser Thr Leu Ile Leu
5 10 15
gtt tta ctt cta tct tta ttt cca aca gct ctt gca gca gaa gga aat 573
Val Leu Leu Leu Ser Leu Phe Pro Thr Ala Leu Ala Ala Glu Gly Asn
20 25 30
acg cgc gaa gac aat ttt gat cat tta tta ggt aat gaa aat gtt aaa 621
Thr Arg Glu Asp Asn Phe Asp His Leu Leu Gly Asn Glu Asn Val Lys
35 40

Taaagaatca ggagagcact tcgggttact cccgtatttt tttggaatgt tttcgaaagc 420
Actttcggta ttttagttat ttgactcaat taagancgat aattaggagg taat atg 477
Met
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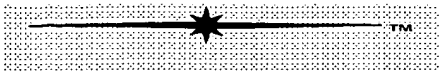

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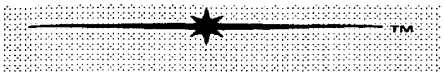

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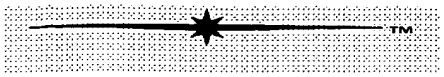

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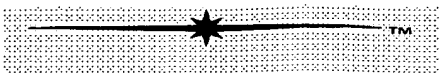

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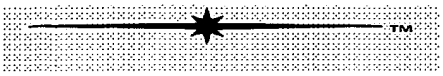

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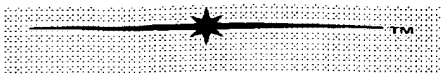

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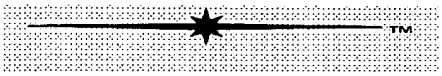

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Met Leu

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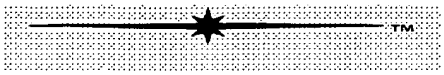

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Ctg cat cag ctt tta att ttt gaa gga tga tcg cag aaa gtt ctt gca 346
Leu His Gln Leu Leu Ile Phe Glu Gly(Trp)Ser Gln Lys Val Leu Ala
5 10 15
Gca gaa gga aac act cgt gaa gac aat ttt aaa cat tta tta ggt aat 394

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gtc gat gga caa atg aca tta gta gat caa cat gga gaa aaa att caa 490
Val Asp Gly Gln Met Thr Leu Val Asp Gln His Gly Glu Lys Ile Gln
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Ala Glu Gly Asn Thr Arg Glu Asp Asn Phe Lys His Leu Leu Gly Asn
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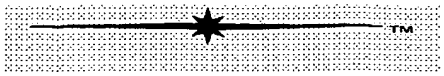

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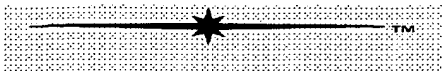

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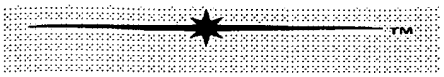

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Ser 215 220
215 225
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230 230 235
235 240 240
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245 255
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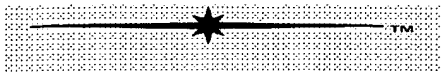

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325 330 335

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375 380 385

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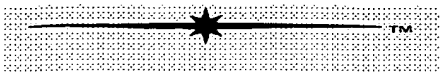

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460 465

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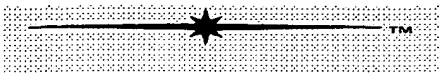

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Gly Leu Thr Ile Thr Gly Glu Asp Ala Pro Ser Leu Glu Ala Ile Ala
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530 535 540 545

Met Asp Val Ile Val Asp Glu Pro Thr Thr Val Ala Ile Ala Ala Ile
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Pro Gln Ser Ser Lys Ser Gly Trp Ala Asn Pro Glu Arg Ala Val Arg
485 490 495
Gtg aac gcg gaa gat ttt gtc cag caa acg gac ggt aag tat aaa gct 1834
Val Asn Ala Glu Asp Phe Val Gln Gln Thr Asp Gly Lys Tyr Lys Ala
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515 520 525 530
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565 570 575

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580 585 590

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Gly Glu Ser Gly Val Lys Thr Ala Leu Thr Ile Glu Glu Ala Asn Gly

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595 600 605 610

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Tct aac gcg tta tca tgg gaa ttt gga tac cca gaa gta aaa cct agt 2170
Ser Asn Ala Leu Ser Trp Glu Phe Gly Tyr Pro Glu Val Lys Pro Ser

Phe Gly Tyr Pro Glu Val Lys Pro
Ser

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620 625 625
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2218 Asp Asn Trp Ala Thr Ala Pro Arg Leu Asp Phe
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630
635 640

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Val Arg Gly Glu Asn Asp Tyr Val Phe Tyr Leu Asp Pro
Thr Phe Asp Phe Tyr Leu Asp 645 650
Pro 655
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2314

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660 665 670
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725 730 735
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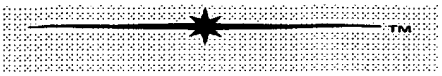

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【００４０】

[0040]

<210> 9
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<213> Artificial Sequence
<220><223> designed DNA based on N131a cellulase
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Agataaactc agaccttgtt gg 22

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[0041]

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<400> 11

gaaggaaaca ctcgtgaaga c 21

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<213> Artificial Sequence
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Gaaggaaaca ctcgtgaaga c 21

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<400> 12

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<213> Artificial Sequence
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<400> 13

[0044]
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<400> 13

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<210> 14
<211> 24
<212> DNA

<213> Artificial Sequence
<220><223> designed DNA based on S237 cellulase
<400> 14

aacagaatga tgttgttcat attg 24

[0045]

<210> 14
<211> 24
<212> DNA

<213> Artificial Sequence
<220><223> designed DNA based on S237 cellulase
<400> 14

Aacagaatga tgttgttcat attg 24

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<210> 15
<211> 21
<212> DNA

<213> Artificial Sequence
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<400> 15

ggtttatatc actatgaagt g 21

[0046]

<210> 15
<211> 21
<212> DNA

<213> Artificial Sequence
<220><223> designed DNA based on S237 cellulase
<400> 15

Ggtttatatc actatgaagt g 21

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<210> 16
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<212> DNA

<213> Artificial Sequence
<220><223> designed DNA based on S237 cellulase

[0047]

<210> 16
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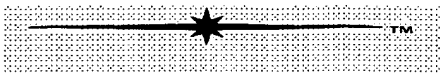
<213> Artificial Sequence
<220><223> designed DNA based on S237 cellulase



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<400> 17

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[0048]

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<210> 18
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tctcgataat aatccctagc gccg 20

[0049]

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<210> 19
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<213> Artificial Sequence
<220><223> designed DNA based on S131b cellulase and

[0050]

<210> 19
<211> 40
<212> DNA

<213> Artificial Sequence
<220><223> designed DNA based on S131b cellulase and KSM-64 cellulase

KSM-64 cellulase
<400> 19

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Agcacaagcc gaaggaaaca ctcgtgaaga caattttaaa 40

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<213> Artificial Sequence
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[0051]
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【００５２】
<210> 21
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<213> Artificial Sequence
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<400> 21

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[0052]
<210> 21
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<213> Artificial Sequence
<220><223> designed DNA based on KSM-64 cellulase
<400> 21

Ttccagatct acttaccat t ttagagtcaa aag 33

【００５３】
<210> 22
<211> 25
<212> DNA

[0053]
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<211> 25
<212> DNA

<213> Artificial Sequence
<220><223> designed DNA based on S131b cellulase and KSM-64 cellulase
<400> 22

ggcttgtgct ggtcgaccca actgc 25

<213> Artificial Sequence
<220><223> designed DNA based on S131b cellulase and KSM-64 cellulase
<400> 22

Ggcttgtgct ggtcgaccca actgc 25

【図面の簡単な説明】

[BRIEF DESCRIPTION OF THE DRAWINGS]

【図１】
本発明のアルカリセルラーゼ（Ｎ１３１ａ）活性に及ぼすｐＨの影響を示す図である。

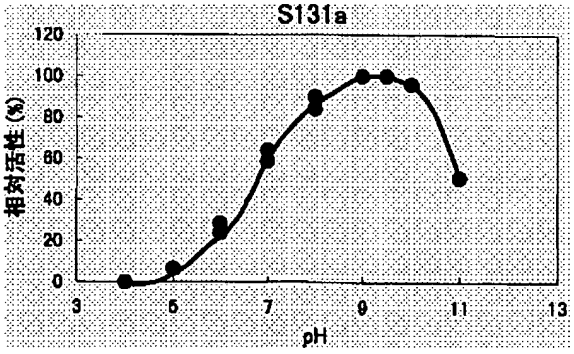
[FIG. 1]
It is the figure showing the influence of pH which affects the alkali cellulase (N131a) activity of this invention.

【図２】
本発明のアルカリセルラーゼ（Ｎ１３１ｂ）活性に及ぼすｐＨの影響を示す図である。

[FIG. 2]
It is the figure showing the influence of pH which affects the alkali cellulase (N131b) activity of this invention.

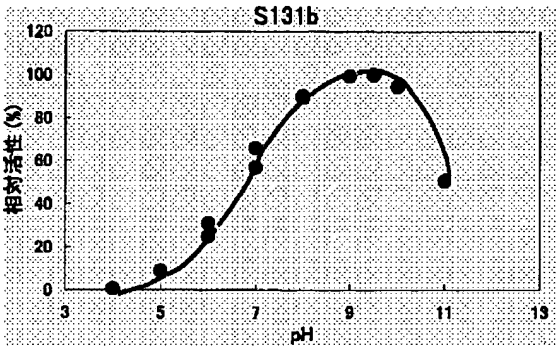
【図１】

[FIG. 1]



相対活性: Relative activity

【図２】 [FIG. 2]



相対活性: Relative activity

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